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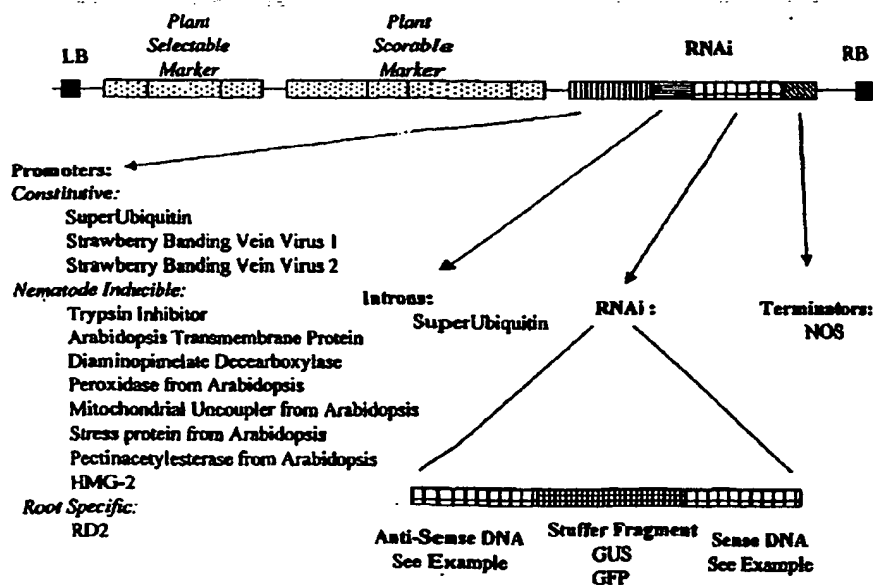
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[Continued on next page]

(54) Title: MATERIALS AND METHODS FOR THE CONTROL OF NEMATODES



(57) Abstract: The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides RNAi molecules, polynucleotide sequences, and methods of using these sequences in nematode control.

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## DESCRIPTION

### MATERIALS AND METHODS FOR THE CONTROL OF NEMATODES

#### Background of the Invention

[0001] Plant parasitic nematodes, such as root-knot nematodes (*Meloidogyne* species) and cyst nematodes (*Globodera* and *Heterodera*), attack nearly every food crop, and are among the world's most damaging agricultural pests. For example, root-knot nematodes parasitize more than 2,000 plant species from diverse plant families and represent a tremendous threat to crop production world-wide. These biotrophic pathogens have evolved highly specialized and complex feeding relationships with their hosts.

[0002] Nematodes cause millions of dollars of damage each year to turf grasses, ornamental plants, and food crops. Efforts to eliminate or minimize damage caused by nematodes in agricultural settings have typically involved the use of soil fumigation with materials such as chloropicrin, methyl bromide, and dazomet, which volatilize to spread the active ingredient throughout the soil. Such fumigation materials can be highly toxic and may create an environmental hazard. Various non-fumigant chemicals have also been used, but these too create serious environmental problems and can be highly toxic to humans.

[0003] Some research articles have been published concerning the effects of  $\delta$ -endotoxins from *B. thuringiensis* species on the viability of nematodes. See, for example, Bottjer, Bone and Gill ([1985] *Experimental Parasitology* 60:239-244); Ignoffo and Dropkin (Ignoffo, C.M., Dropkin, V.H. [1977] *J. Kans. Entomol. Soc.* 50:394-398); and Ciordia, H. and W.E. Bizzell ([1961] *Jour. of Parasitology* 47:41 [abstract]). Several patents have issued describing the control of nematodes with *B.t.* See, for example, U.S. Patent Nos. 4,948,734; 5,093,120; 5,281,530; 5,426,049; 5,439,881; 5,236,843; 5,322,932; 5,151,363; 5,270,448; 5,350,577; 5,667,993; and 5,670,365. The development of resistance by insects to *B.t.* toxins is one obstacle to the successful use of such toxins.

[0004] The pesticidal activity of avermectins is well known. The avermectins are disaccharide derivatives of pentacyclic, 16-membered lactones. They can be divided into four major compounds: A<sub>1a</sub>, A<sub>2a</sub>, B<sub>1a</sub>, and B<sub>2a</sub>; and four minor compounds: A<sub>1b</sub>, A<sub>2b</sub>, B<sub>1b</sub>, and B<sub>2b</sub>. The isolation and purification of these compounds is also described in U.S. Patent No. 4,310,519, issued January 12, 1982. Avermectin B<sub>2a</sub> is active against the root-knot nematode, *Meloidogyne incognita*. It is reported to be 10-30 times as potent as commercial contact nematicides when incorporated into soil at 0.16-0.25 kg/ha (Boyce Thompson Institute for Plant Research 58th Annual Report [1981]; Putter, I. *et al.* [1981] "Avermectins: Novel Insecticides, Acaracides, and Nematicides from a Soil Microorganism," *Experientia* 37:963-964). Avermectin B<sub>2a</sub> is not toxic to tomatoes or cucumbers at rates of up to 10 kg/ha.

[0005] Fatty acids are a class of natural compounds which occur abundantly in nature and which have interesting and valuable biological activities. Tarjan and Cheo (Tarjan, A.C., P.C. Cheo [1956] "Nematocidal Value of Some Fatty Acids," Bulletin 332, Contribution 884, Agricultural Experiment Station, University of Rhode Island, Kingston, 41 pp.) report the activity of certain fatty acids against nematodes. In 1977 Sitaramaiah and Singh (Sitaramaiah, K., R.S. Singh [1977] *Indian J. Nematol.* 7:58-65) also examined the response of nematodes to fatty acids. The results of these tests with short chain acids were equivocal, showing nematode-inhibitory action in some instances and stimulatory activity in other instances. Phytotoxicity of these acids was observed at higher concentrations. The short chain fatty acids were also examined by Malik and Jairajpuri (Malik, Z., M.S. Jairajpuri [1977] *Nematol. medit.* 12:73-79), who observed nematode toxicity at high concentrations of the fatty acids.

[0006] Notwithstanding the foregoing (some of the limitations of and problems associated with these approaches are discussed above), there is a need for safe and effective alternatives for controlling nematodes.

[0007] One method for disrupting normal cellular processes is by the use double-stranded interfering RNA (RNAi), or RNA-mediated interference (RNAi). When RNAi corresponding to a sense and antisense sequence of a target mRNA is introduced into a cell, the targeted mRNA is degraded and protein translation of that message is stopped. Although not yet fully understood, the mechanism of this post-transcriptional gene

silencing appears to be at least partially due to the generation of small RNA molecules, about 21 - 25 nucleotides in length, that correspond to the sense and antisense pieces of the RNAi introduced into the cell (Bass, B. L. [2000] "Double-stranded RNA as a template for gene silencing" *Cell* 101:235-238).

[0008] The specificity of this gene silencing mechanism appears to be extremely high, blocking expression only of targeted genes, while leaving other genes unaffected. A recent example of the use of RNAi; to inhibit genetic function in plants used *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* (Chuang, C.-F. and E. M. Meyerowitz [2000] "Specific and heritable genetic interference by double-stranded RNA in *Arabidopsis thaliana*" *Proc. Natl. Acad. Sci. USA* 97:4985-4990). Chuang *et al.* describe the construction of vectors delivering variable levels of RNAi targeted to each of four genes involved in floral development. Severity of abnormal flower development varied between transgenic lines. For one of the genes, AGAMOUS (AG), a strong correlation existed between declining accumulation of mRNA and increasingly severe phenotypes, suggesting that AG-specific endogenous mRNA is the target of RNAi.

#### Brief Summary of the Invention

[0009] The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides polynucleotide sequences that encode nematode genes, RNAi that selectively targets mRNA transcripts of these essential nematode genes, and methods of using these sequences in nematode control strategies. Such sequences for use according to the subject invention are summarized in Appendix 1. RNAi molecules disclosed herein can be used to inhibit the expression of one or more of these genes in nematodes.

### Brief Description of the Drawings

[00010] **Figure 1:** Modular Binary Construct System (MBCS): A series of six, 8-base cutter restriction enzyme sites has been placed between the left and right T<sub>i</sub> borders of a previously created kan<sup>R</sup>/tet<sup>R</sup> binary plasmid.

[00011] **Figure 2:** An exemplary shuttle vector created for cloning of useful DNA fragments by containing the multi-cloning site (MCS) of a modified Bluescript plasmid flanked by 8-base restriction sites.

[00012] **Figure 3:** An exemplary shuttle vector with exemplary inserts.

[00013] **Figure 4:** A suggested RNAi binary vector with exemplary inserts.

[00014] **Figure 5:** Exemplary selectable markers for MBCS.

[00015] **Figure 6:** Exemplary scorable markers for MCBS.

[00016] **Figure 7:** Exemplary RNAi binary vector.

[00017] **Figure 8:** Exemplary RNAi shuttle vector.

### Brief Description of the Sequences

[00018] Brief Description of the Sequences can be found in Appendix I.

### Detailed Disclosure of the Invention

[00019] The subject invention provides novel methods and compositions for controlling nematodes. More specifically, the subject invention provides polynucleotide sequences and methods of using these sequences in nematode control strategies. A preferred method for controlling nematodes according to the subject invention provides materials and methods for controlling nematodes by using double-stranded interfering RNA (RNAi), or RNA-mediated interference (RNAi). The terms RNAi and RNAi are used interchangeably herein unless otherwise noted.

[00020] In one embodiment of the invention, RNAi molecules are provided which are useful in methods of killing nematodes and/or inhibiting their growth, development, parasitism or reproduction. RNAi molecules of the invention are also useful for the regulation of levels of specific mRNA in nematodes.

[00021] dsRNA (RNAi) typically comprises a polynucleotide sequence identical to a target gene (or fragment thereof) linked directly, or indirectly, to a polynucleotide

may be chemically or enzymatically synthesized by manual or automated reactions. The RNA may be synthesized by a cellular RNA polymerase or a bacteriophage RNA polymerase (e.g., T3, T7, SP6). The use and production of an expression construct are known in the art (see, for example, WO 97/32016; U.S. Pat. Nos. 5,593,874; 5,698,425; 5,712,135; 5,789,214; and 5,804,693; and the references cited therein). If synthesized chemically or by *in vitro* enzymatic synthesis, the RNA may be purified prior to introduction into the cell. For example, RNA can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography, or a combination thereof. Alternatively, the RNA may be used with no or a minimum of purification to avoid losses due to sample processing. The RNA may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to promote annealing, and/or stabilization of the duplex strands.

[00025] Preferably and most conveniently, RNAi can be targeted to an entire polynucleotide sequence of a gene set forth herein. Preferred RNAi molecules of the instant invention are highly homologous or identical to the polynucleotides summarized in Appendix 1. The homology is preferably greater than 90% and is most preferably greater than 95%.

[00026] Fragments of genes can also be targeted. These fragments are typically in the approximate size range of about 20 nucleotides. Thus, targeted fragments are preferably at least about 15 nucleotides. In certain embodiments, the gene fragment targeted by the RNAi molecule is about 20-25 nucleotides in length. However, other size ranges can also be used. For example, using a *C. elegans* microinjection assay, RNAi "fragments" of about 60 nucleotides with between 95 and 100% identity (to a nematode gene) were determined to cause excellent inhibition.

[00027] Thus, RNAi molecules of the subject invention are not limited to those that are targeted to the full-length polynucleotide or gene. The nematode gene product can be inhibited with a RNAi molecule that is targeted to a portion or fragment of the exemplified polynucleotides; high homology (90-95%) or identity is also preferred, but not necessarily essential, for such applications.

[00028] The polynucleotide sequences identified in Appendix A and shown in the Sequence ID listing are from genes encoding nematode proteins having the functions

sequence complementary to the sequence of the target gene (or fragment thereof). The dsRNA may comprise a polynucleotide linker (stuffer) sequence of sufficient length to allow for the two polynucleotide sequences to fold over and hybridize to each other; however, a linker sequence is not necessary. The linker (stuffer) sequence is designed to separate the antisense and sense strands of RNAi significantly enough to limit the effects of steric hindrances and allow for the formation of dsRNA molecules.

[00022] RNA containing a nucleotide sequence identical to a fragment of the target gene is preferred for inhibition; however, RNA sequences with insertions, deletions, and point mutations relative to the target sequence can also be used for inhibition. Sequence identity may be optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, *Sequence Analysis Primer*, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (e.g., University of Wisconsin Genetic Computing Group). Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a fragment of the target gene transcript.

[00023] As disclosed herein, 100% sequence identity between the RNA and the target gene is not required to practice the present invention. Thus the invention has the advantage of being able to tolerate sequence variations that might be expected due to genetic mutation, strain polymorphism, or evolutionary divergence.

[00024] RNA may be synthesized either *in vivo* or *in vitro*. Endogenous RNA polymerase of the cell may mediate transcription *in vivo*, or cloned RNA polymerase can be used for transcription *in vivo* or *in vitro*. For transcription from a transgene *in vivo* or an expression construct, a regulatory region (e.g., promoter, enhancer, silencer, splice donor and acceptor, polyadenylation) may be used to transcribe the RNA strand (or strands). Inhibition may be targeted by specific transcription in an organ, tissue, or cell type; stimulation of an environmental condition (e.g., infection, stress, temperature, chemical inducers); and/or engineering transcription at a developmental stage or age. The RNA strands may or may not be polyadenylated; the RNA strands may or may not be capable of being translated into a polypeptide by a cell's translational apparatus. RNA



shown in Appendix 1. The genes exemplified herein are representative of particular classes of proteins which are preferred targets for disruption according to the subject invention. These classes of proteins include, for example, proteins involved in ribosome assembly; neurotransmitter receptors and ligands; electron transport proteins; metabolic pathway proteins; and protein and polynucleotide production, folding, and processing proteins.

[00029] Genetic regulatory sequences, such as promoters, enhancers, and terminators, can be used in genetic constructs to practice the subject invention. Such constructs themselves can also be used for nematode control. Various constructs can be used to achieve expression in specific plant tissues (by using root specific promoters, for example) and/or to target specific nematode tissues (by using targeting elements or adjacent targeting sequences, for example).

[00030] In a specific embodiment of the subject invention, plant cells, preferably root cells, are genetically modified to produce at least one RNAi that is designed to be taken up by nematodes during feeding to block expression (or the function of) of a target gene. As is known in the art, RNAi can target and reduce (and, in some cases, prevent) the translation of a specific gene product. RNAi can be used to reduce or prevent message translation in any tissue of the nematode because of its ability to cross tissue and cellular boundaries. Thus, RNAi that is contacted with a nematode by soaking, injection, or consumption of a food source will cross tissue and cellular boundaries. RNAi can also be used as an epigenetic factor to prevent the proliferation of subsequent generations of nematodes.

[00031] Nematode polynucleotide sequences disclosed herein demonstrate conserved nucleotide motifs among different nematode genera. Conserved nucleotide motifs strongly suggest that these sequences are associated with viability and/or parasitism and are functionally conserved and expressed in both *Meloidogyne incognita* (root-knot nematode) and *Globodera rostochiensis* and *Globodera pallida* (potato cyst nematodes). The use of these polynucleotides, and RNAi inhibitors thereof, is advantageous because such RNAi can be designed to have broad RNAi specificity and are thus useful for controlling a large number of plant parasitic nematodes *in planta*. Because the genes identified in this disclosure are associated with nematode survival

heritable inhibition of gene expression (Sarkissian, M., H. Tabara and C. C. Mello [1999] "A mut-6 screen for RNAi deficient mutants" International Worm Meeting, Madison, WI, abstract 741; Timmons, L. and A. Fire [1998] "Specific interference by ingested dsRNA" *Nature* 395:854; WO 99/32619, hereby incorporated by reference in its entirety).

[00035] Accordingly, one aspect of the instant invention is directed to the control of nematodes comprising contacting nematodes with compositions comprising RNAi molecules specific to the nematode genes disclosed herein. The contacting step may include soaking the nematodes in a solution containing RNAi molecules, feeding nematodes RNAi molecules contained in microbes or plant cells upon which the nematode feeds, or injecting nematodes with RNAi. Nematodes can also be "contacted" and controlled by RNAi expressed in plant tissues that would be consumed, ingested, or frequented by nematodes.

[00036] The RNAi molecules provided to the nematodes may be specific to a single gene. A "cocktail" of RNAi molecules specific to various segments of a single gene can also be used. In addition, a "multigene cocktail" of RNAi molecules specific to two or more genes (or segments thereof) may be applied to the nematodes according to the subject invention.

[00037] In addition to RNAi uptake mediated by transgenic plants, nematodes can be directly transformed with RNAi constructs of cDNAs encoding secretory or other essential proteins to reduce expression of the corresponding gene. The transgenic animals can be assayed for inhibition of gene product using immunoassays or for reduced virulence on a host. Progeny of affected worms can also be assayed by similar methods.

[00038] Procedures that can be used for the preparation and injection of RNAi include those detailed by Fire *et al.*, (1998; <ftp://ciw1.ciwemb.edu>). Root-knot nematodes can be routinely monoxenically cultured on *Arabidopsis thaliana* roots growing on Gamborg's B-5/Gelrite® media. This nematode-host pathosystem is ideally suited for these microinjection experiments since limited root galling results in the parasitic stages (late J2 through adult females) developing outside of the root for easy accessibility for injecting. Another advantage is the parthenogenic reproduction of root-knot nematodes, which makes fertilization by males unnecessary for egg production. The RNAi can be injected into the body cavity of parasitic stages of root-knot nematodes

[00041] Another assay is designed to determine the effect of the RNAi on reducing the virulence of J2 progeny of the injected females. Egg masses from injected females can be transferred singly to *A. thaliana* plates to assess the ability of the transgenic J2 to infect roots. The J2 hatching from the eggs transferred to the plates can be monitored; after 25 days the number of galls with egg laying females can be recorded. The *A. thaliana* roots can also be stained with acid fuchsin to enumerate the number of nematodes in the roots. Egg masses from nematodes injected only with the injection buffer can be handled similarly and used as controls. The treatments can be replicated, and the root infection data can be analyzed statistically. These experiments can be used to assess the importance of the target genes in root-knot nematode's virulence or viability. By staining the J2 progeny of the injected females with the antibodies, it can be determined whether RNAi blocks expression of the targeted gene.

[00042] Additional uses of polynucleotides. The polynucleotide sequences exemplified herein can be used in a variety of ways. These polynucleotides can be used in assays for additional polynucleotides and additional homologous genes, and can be used in tracking the quantitative and temporal expression of parasitism genes in nematodes. These polynucleotides can be cloned into microbes for production and isolation of their gene products. Among the many uses of the isolated gene product is the development of additional inhibitors and modifiers. The protein products of the subject polynucleotides can also be used as diagnostic tools. For example, proteins encoded by the parasitism genes, as identified herein, can be used in large scale screenings for additional peptide inhibitors. The use of peptide phage display screening is one method that can be used in this regard. Thus, the subject invention also provides new biotechnological strategies for managing nematodes under sustainable agricultural conditions.

[00043] Antisense technologies can also be used for phytopathogenic nematode control. Antisense technology can be used to interfere with expression of the disclosed endogenous nematode genes. Antisense technology can also be used to alter the components of plants used as targets by the nematodes. For example, the transformation of a plant with the reverse complement of an endogenous gene encoded by a polynucleotide exemplified herein can result in strand co-suppression and gene silencing

feeding on *A. thaliana* roots using microinjection. Control nematodes can be injected in parallel with only buffer or an unrelated RNAi. Injected nematodes can be monitored for egg production, and the eggs can be collected for the assays described below. Female root-knot nematodes will typically survive and lay more than 250 eggs following 1  $\mu$ l injection of buffer.

[00039] Alternatively, methods are available for microinjecting materials directly into the plant root cells upon which nematodes feed: giant cells or syncytial cells (Böckenhoff, A. and F.M.W. Grundler [1994] "Studies on the nutrient uptake by the beet cyst nematode *Heterodera schachtii* by *in situ* microinjection of fluorescent probes into the feeding structures in *Arabidopsis thaliana*" *Parasitology* 109:249-254). This provides an excellent test system to screen RNAi molecules for efficacy by directly inhibiting growth and development of the nematode feeding upon the microinjected plant cell, or by reducing fecundity and the ability of said nematode to generate pathogenic or viable progeny.

[00040] There are a number of strategies that can be followed to assay for RNAi gene interference. Inhibition of gene expression by RNAi inhibits the accumulation of the corresponding secretory protein in the esophageal gland cells of transgenic J2 hatched from the eggs produced by the injected nematodes. In the first assay, polyclonal antibodies to the target gene product can be used in immunolocalization studies (Hussey, R. S. [1989] "Monoclonal antibodies to secretory granules in esophageal glands of *Meloidogyne* species" *J. Nematol.* 21:392-398; Borgonie, G, E. van Driessche, C. D. Link, D. de Waele, and A. Coomans [1994] "Tissue treatment for whole mount internal lectin staining in the nematodes *Caenorhabditis elegans*, *Paragrolaimus superbus* and *Acrobeloides maximus*" *Histochemistry* 101:379-384) to monitor the synthesis of the target protein in the gland cells of progeny of the injected nematodes, or in any other nematode tissue that fails to express the essential targeted gene. Interference of endogenous gene activity by the RNAi eliminates binding of the antibodies to secretory granules in the glands, or any other target tissue, of the transgenic nematodes, and can be monitored by these *in situ* hybridization experiments. Control nematodes injected only with the injection buffer can be processed similar to the RNAi treated nematodes.

or inhibition of a target involved in the nematode infection process. Thus, the subject invention includes transgenic plants (which are preferably made nematode-resistant in this manner, and other organisms including microbes and phages) comprising RNAi or antisense molecules specific to any of the polynucleotides identified herein.

[00044] Polynucleotide probes. DNA possesses a fundamental property called base complementarity. In nature, DNA ordinarily exists in the form of pairs of anti-parallel strands, the bases on each strand projecting from that strand toward the opposite strand. The base adenine (A) on one strand will always be opposed to the base thymine (T) on the other strand, and the base guanine (G) will be opposed to the base cytosine (C). The bases are held in apposition by their ability to hydrogen bond in this specific way. Though each individual bond is relatively weak, the net effect of many adjacent hydrogen bonded bases, together with base stacking effects, is a stable joining of the two complementary strands. These bonds can be broken by treatments such as high pH or high temperature, and these conditions result in the dissociation, or "denaturation," of the two strands. If the DNA is then placed in conditions which make hydrogen bonding of the bases thermodynamically favorable, the DNA strands will anneal, or "hybridize," and reform the original double-stranded DNA. If carried out under appropriate conditions, this hybridization can be highly specific. That is, only strands with a high degree of base complementarity will be able to form stable double-stranded structures. The relationship of the specificity of hybridization to reaction conditions is well known. Thus, hybridization may be used to test whether two pieces of DNA are complementary in their base sequences. It is this hybridization mechanism which facilitates the use of probes of the subject invention to readily detect and characterize DNA sequences of interest.

[00045] The specifically exemplified polynucleotides of the subject invention can themselves be used as probes. Additional polynucleotide sequences can be added to the ends of (or internally in) the exemplified polynucleotide sequences so that polynucleotides that are longer than the exemplified polynucleotides can also be used as probes. Thus, isolated polynucleotides comprising one or more of the exemplified sequences are within the scope of the subject invention. Polynucleotides that have less nucleotides than the exemplified polynucleotides can also be used and are contemplated within the scope of the present invention. For example, for some purposes, it might be

useful to use a conserved sequence from an exemplified polynucleotide wherein the conserved sequence comprises a portion of an exemplified sequence. Thus, polynucleotides of the subject invention can be used to find additional, homologous (wholly or partially) genes.

[00046] Probes of the subject invention may be composed of DNA, RNA, or PNA (peptide nucleic acid). The probe will normally have at least about 10 bases, more usually at least about 17 bases, and may have about 100 bases or more. Longer probes can readily be utilized, and such probes can be, for example, several kilobases in length. The probe sequence is designed to be at least substantially complementary to a portion of a gene encoding a protein of interest. The probe need not have perfect complementarity to the sequence to which it hybridizes. The probes may be labeled utilizing techniques that are well known to those skilled in this art.

[00047] One approach for the use of the subject invention as probes entails first identifying DNA segments that are homologous with the disclosed nucleotide sequences using, for example, Southern blot analysis of a gene bank. Thus, it is possible, without the aid of biological analysis, to know in advance the probable activity of many new polynucleotides, and of the individual gene products expressed by a given polynucleotide. Such an analysis provides a rapid method for identifying commercially valuable compositions.

[00048] One hybridization procedure useful according to the subject invention typically includes the initial steps of isolating the DNA sample of interest and purifying it chemically. Either lysed nematodes or total fractionated nucleic acid isolated from nematodes can be used. Cells can be treated using known techniques to liberate their DNA (and/or RNA). The DNA sample can be cut into pieces with an appropriate restriction enzyme. The pieces can be separated by size through electrophoresis in a gel, usually agarose or acrylamide. The pieces of interest can be transferred to an immobilizing membrane.

[00049] The particular hybridization technique is not essential to the subject invention. As improvements are made in hybridization techniques, they can be readily applied.

and/or parasitism, RNAi inhibition of these genes (arising from contacting nematodes with compositions comprising RNAi molecules) prevents and/or reduces parasitic nematode growth, development, and or parasitism.

[00032] Methods of the subject invention include the transformation of plant cells with genes or polynucleotides of the present invention, which can be used to produce nematode inhibitors or RNAi in the plants. In one embodiment, the transformed plant or plant tissue can express RNAi molecules encoded by the gene or polynucleotide sequence introduced into the plant. Other nematode inhibitors contemplated by the invention include antisense molecules specific to the polynucleotide sequences disclosed herein. The transformation of plants with genetic constructs disclosed herein can be accomplished using techniques well known to those skilled in the art and can involve modification of the gene(s) to optimize expression in the plant to be made resistant to nematode infection and infestation. Furthermore, it is known in the art that many tissues of the transgenic plants (such as the roots) can be targeted for transformation.

[00033] RNA-mediated interference (RNAi) of gene expression. Several aspects of root-knot nematode biology make classical genetic studies difficult with this organism. Since root-knot nematodes reproduce by obligatory mitotic parthenogenesis, the opportunity to perform genetic crosses is not available. Microinjection of RNAi can be used to manipulate gene expression in *C. elegans* (Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello. [1998] "Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*" *Nature* 391:806-811). Microinjecting (into adult nematodes) RNAi can turn off specific genes in progeny worms complementary to the coding region of the genes. Moreover, gene inhibition occurs in progeny when RNAi is injected into the body cavity of the adult, indicating the ability of the RNAi to cross cellular boundaries. This RNAi injection method provides a molecular genetic tool that allows for analysis of gene function in root-knot nematodes.

[00034] RNAi can be taken up by *C. elegans* by simply soaking the nematodes in a solution RNAi. This results in targeted inhibition of gene expression in the nematode (Maeda, I., Y. Kohara, M. Yamamoto and A. Sugimoto [1999] "RNAi screening with a non-redundant cDNA set" International Worm Meeting, Madison, WI, abstract 565). Nematodes fed *E. coli* expressing RNAi also demonstrate targeted and

[00050] The probe and sample can then be combined in a hybridization buffer solution and held at an appropriate temperature until annealing occurs. Thereafter, the membrane is washed free of extraneous materials, leaving the sample and bound probe molecules typically detected and quantified by autoradiography and/or liquid scintillation counting. As is well known in the art, if the probe molecule and nucleic acid sample hybridize by forming a strong non-covalent bond between the two molecules, it can be reasonably assumed that the probe and sample are essentially identical or very similar. The probe's detectable label provides a means for determining in a known manner whether hybridization has occurred.

[00051] In the use of the nucleotide segments as probes, the particular probe is labeled with any suitable label known to those skilled in the art, including radioactive and non-radioactive labels. Typical radioactive labels include  $^{32}\text{P}$ ,  $^{35}\text{S}$ , or the like. Non-radioactive labels include, for example, ligands such as biotin or thyroxine, as well as enzymes such as hydrolases or peroxidases, or the various chemiluminescers such as luciferin, or fluorescent compounds like fluorescein and its derivatives. In addition, the probes can be made inherently fluorescent as described in International Application No. WO 93/16094.

[00052] Various degrees of stringency of hybridization can be employed. The more stringent the conditions, the greater the complementarity that is required for duplex formation. Stringency can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Preferably, hybridization is conducted under moderate to high stringency conditions by techniques well known in the art, as described, for example, in Keller, G.H., M.M. Manak (1987) *DNA Probes*, Stockton Press, New York, NY., pp. 169-170.

[00053] As used herein "moderate to high stringency" conditions for hybridization refers to conditions that achieve the same, or about the same, degree of specificity of hybridization as the conditions "as described herein." Examples of moderate to high stringency conditions are provided herein. Specifically, hybridization of immobilized DNA on Southern blots with  $^{32}\text{P}$ -labeled gene-specific probes was performed using standard methods (Maniatis *et al.*). In general, hybridization and subsequent washes were carried out under moderate to high stringency conditions that



allowed for detection of target sequences with homology to sequences exemplified herein. For double-stranded DNA gene probes, hybridization was carried out overnight at 20-25 ° C below the melting temperature ( $T_m$ ) of the DNA hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula from Beltz *et al.* (1983):

[00054]  $T_m = 81.5^\circ\text{C} + 16.6 \log[\text{Na}^+] + 0.41(\%G+C) - 0.61(\%\text{formamide}) - 600/\text{length of duplex in base pairs}.$

Washes are typically carried out as follows:

- (1) Twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash).
- (2) Once at  $T_m - 20^\circ\text{C}$  for 15 minutes in 0.2X SSPE, 0.1% SDS (moderate stringency wash).

[00055] For oligonucleotide probes, hybridization was carried out overnight at 10-20°C below the melting temperature ( $T_m$ ) of the hybrid in 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA.  $T_m$  for oligonucleotide probes was determined by the following formula from Suggs *et al.* (1981):

[00056]  $T_m (^\circ\text{C}) = 2(\text{number T/A base pairs}) + 4(\text{number G/C base pairs})$

[00057] Washes were typically carried out as follows:

- [00058] (1) Twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash).
- [00059] (2) Once at the hybridization temperature for 15 minutes in 1X SSPE, 0.1% SDS (moderate stringency wash).

[00060] In general, salt and/or temperature can be altered to change stringency. With a labeled DNA fragment of greater than about 70 or so bases in length, the following conditions can be used:

- |           |                                |
|-----------|--------------------------------|
| Low:      | 1 or 2X SSPE, room temperature |
| Low:      | 1 or 2X SSPE, 42°C             |
| Moderate: | 0.2X or 1X SSPE, 65°C          |
| High:     | 0.1X SSPE, 65°C.               |

[00061] Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid, and, as noted above, a certain degree of mismatch

can be tolerated. Therefore, polynucleotide sequences of the subject invention include mutations (both single and multiple), deletions, and insertions in the described sequences, and combinations thereof, wherein said mutations, insertions, and deletions permit formation of stable hybrids with a target polynucleotide of interest. Mutations, insertions, and deletions can be produced in a given polynucleotide sequence using standard methods known in the art. Other methods may become known in the future.

[00062] The mutational, insertional, and deletional variants of the polynucleotide sequences of the invention can be used in the same manner as the exemplified polynucleotide sequences so long as the variants have substantial sequence similarity with the original sequence. As used herein, substantial sequence similarity refers to the extent of nucleotide similarity that is sufficient to enable the variant polynucleotide to function in the same capacity as the original sequence. Preferably, this similarity is greater than 50%; more preferably, this similarity is greater than 75%; and most preferably, this similarity is greater than 90%. The degree of similarity needed for the variant to function in its intended capacity will depend upon the intended use of the sequence. It is well within the skill of a person trained in this art to make mutational, insertional, and deletional mutations that are designed to improve the function of the sequence or otherwise provide a methodological advantage.

[00063] PCR technology. Polymerase Chain Reaction (PCR) is a repetitive, enzymatic, primed synthesis of a nucleic acid sequence. This procedure is well known and commonly used by those skilled in this art (see U.S. Patent Nos. 4,683,195; 4,683,202; and 4,800,159; Saiki *et al.*, 1985). PCR is based on the enzymatic amplification of a DNA fragment of interest that is flanked by two oligonucleotide primers that hybridize to opposite strands of the target sequence. The primers are oriented with the 3' ends pointing towards each other. Repeated cycles of heat denaturation of the template, annealing of the primers to their complementary sequences, and extension of the annealed primers with a DNA polymerase result in the amplification of the segment defined by the 5' ends of the PCR primers. Since the extension product of each primer can serve as a template for the other primer, each cycle essentially doubles the amount of DNA fragment produced in the previous cycle. This results in the exponential accumulation of the specific target fragment, up to several million-fold in a

few hours. By using a thermostable DNA polymerase such as *Taq* polymerase, which is isolated from the thermophilic bacterium *Thermus aquaticus*, the amplification process can be completely automated. Other enzymes that can be used are known to those skilled in the art.

[00064] The polynucleotide sequences of the subject invention (and portions thereof such as conserved regions and portions that serve to distinguish these sequences from previously-known sequences) can be used as, and/or used in the design of, primers for PCR amplification. In performing PCR amplification, a certain degree of mismatch can be tolerated between primer and template. Therefore, mutations, deletions, and insertions (especially additions of nucleotides to the 5' end) of the exemplified polynucleotides can be used in this manner. Mutations, insertions and deletions can be produced in a given primer by methods known to an ordinarily skilled artisan.

[00065] The polynucleotide sequences of the instant invention may be "operably linked" to regulatory sequences such as promoters and enhancers. Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is "operably linked" to DNA encoding a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is "operably linked" to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is "operably linked" to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[00066] Polynucleotides and proteins. Polynucleotides of the subject invention can be defined according to several parameters. One characteristic is the biological activity of the protein products as identified herein. The proteins and genes of the subject invention can be further defined by their amino acid and nucleotide sequences. The sequences of the molecules can be defined in terms of homology to certain exemplified sequences as well as in terms of the ability to hybridize with, or be amplified by, certain

exemplified probes and primers. Additional primers and probes can readily be constructed by those skilled in the art such that alternate polynucleotide sequences encoding the same amino acid sequences can be used to identify and/or characterize additional genes. The proteins of the subject invention can also be identified based on their immunoreactivity with certain antibodies.

[00067] The polynucleotides and proteins of the subject invention include portions, fragments, variants, and mutants of the full-length sequences as well as fusions and chimerics, so long as the encoded protein retains the characteristic biological activity of the proteins identified herein. As used herein, the terms "variants" or "variations" of genes refer to nucleotide sequences that encode the same proteins or which encode equivalent proteins having equivalent biological activity. As used herein, the term "equivalent proteins" refers to proteins having the same or essentially the same biological activity as the exemplified proteins.

[00068] It will be apparent to a person skilled in this art that genes within the scope of the subject invention can be identified and obtained through several means. The specific genes exemplified herein may be obtained from root-knot nematodes. Genes, or portions or variants thereof, may also be artificially synthesized by, for example, a gene synthesizer.

[00069] Variations of genes may be readily constructed using standard techniques such as site-directed mutagenesis and other methods of making point mutations and by DNA shuffling, for example. In addition, gene and protein fragments can be made using commercially available exonucleases, endonucleases, and proteases according to standard procedures. For example, enzymes such as *Bal31* can be used to systematically cut off nucleotides from the ends of genes. In addition, genes that encode fragments may be obtained using a variety of restriction enzymes. Proteases may be used to directly obtain active fragments of these proteins. Of course, molecular techniques for cloning polynucleotides and producing gene constructs of interest are also well known in the art. *In vitro* evaluation techniques, such as MAXYGEN's "Molecular Breeding" can also be applied to practice the subject invention.

[00070] Other molecular techniques can also be applied using the teachings provided herein. For example, antibodies raised against proteins encoded by

polynucleotides disclosed herein can be used to identify and isolate proteins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the proteins that are conserved and most distinct from other proteins. These antibodies can then be used to specifically identify equivalent proteins by immunoprecipitation, enzyme linked immunosorbent assay (ELISA), or Western blotting. Antibodies to proteins encoded by polynucleotides disclosed herein, or to equivalent proteins, can readily be prepared using standard procedures known in the art. The genes that encode these proteins can be obtained from various organisms.

[00071] Because of the redundancy of the genetic code, a variety of different DNA sequences can encode the amino acid sequences encoded by the polynucleotide sequences disclosed herein. It is well within the skill of a person trained in the art to create these alternative DNA sequences encoding proteins having the same, or essentially the same, amino acid sequence. These variant DNA sequences are within the scope of the subject invention. As used herein, reference to "essentially the same" sequence refers to sequences that have amino acid substitutions, deletions, additions, or insertions that do not materially affect biological activity. Fragments retaining the characteristic biological activity are also included in this definition.

[00072] A further method for identifying genes and polynucleotides (and the proteins encoded thereby) of the subject invention is through the use of oligonucleotide probes. Probes provide a rapid method for identifying genes of the subject invention. The nucleotide segments that are used as probes according to the invention can be synthesized using a DNA synthesizer and standard procedures.

[00073] The subject invention comprises variant or equivalent proteins (and nucleotide sequences coding for equivalent proteins or for inhibitors of the genes encoding such proteins) having the same or similar biological activity of inhibitors or proteins encoded by the exemplified polynucleotides. Equivalent proteins will have amino acid similarity with an exemplified protein (or peptide). The amino acid and/or nucleotide identity will typically be greater than 60%. Preferably, the identity will be greater than 75%. More preferably, the identity will be greater than 80%, and even more preferably greater than 90%. Most preferably, the identity will be greater than 95%. RNAi molecules will also have corresponding identities in these preferred ranges. These

identities are as determined using standard alignment techniques for determining amino acid and/or nucleotide identity. The identity/similarity will be highest in critical regions of the protein or gene including those regions that account for biological activity or that are involved in the determination of three-dimensional configuration that is ultimately responsible for the biological activity. In this regard, certain amino acid substitutions are acceptable and can be expected if these substitutions are in regions which are not critical to activity or are conservative amino acid substitutions which do not affect the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Below is a list of examples of amino acids belonging to various classes

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Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

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[00074] In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not detract from the ability to manage nematode-caused diseases.

[00075] An "isolated" or "substantially pure" nucleic acid molecule or polynucleotide is a polynucleotide that is substantially separated from other polynucleotide sequences which naturally accompany a nucleic acid molecule. The term embraces a polynucleotide sequence which was removed from its naturally occurring environment by the hand of man. This includes recombinant or cloned DNA isolates,

chemically synthesized analogues and analogues biologically synthesized by heterologous systems. An "isolated" or "purified" protein, likewise, is a protein removed from its naturally occurring environment.

[00076] Recombinant hosts. The genes, antisense, and RNAi polynucleotides within the scope of the present invention can be introduced into a wide variety of microbial or plant hosts. Plant cells can be transformed (made recombinant) in this manner. Microbes, for example, can also be used in the application of RNAi molecules of the subject invention in view of the fact that microbes are a food source for nematodes

[00077] There are many methods for introducing a heterologous gene or polynucleotide into a host cell or cells under conditions that allow for stable maintenance and expression of the gene or polynucleotide. These methods are well known to those skilled in the art. Synthetic genes, such as, for example, those genes modified to enhance expression in a heterologous host (such as by preferred codon usage or by the use of adjoining, downstream, or upstream enhancers) that are functionally equivalent to the genes (and which encode equivalent proteins) can also be used to transform hosts. Methods for the production of synthetic genes are known in the art.

[00078] Where the gene or polynucleotide of interest is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, certain host microbes are preferred. Certain microorganism hosts are known to occupy the phytosphere, phylloplane, phyllosphere, rhizosphere, and/or rhizoplane of one or more crops of interest. These microorganisms can be selected so as to be capable of successfully competing in the particular environment (crop and other habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing a polypeptide of interest, and, desirably, provide for improved protection of the protein/peptide from environmental degradation and inactivation.

[00079] A large number of microorganisms is known to inhabit the phylloplane (the surface of the plant leaves) and/or the rhizosphere (the soil surrounding plant roots) of a wide variety of important crops. These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, e.g., genera *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*, *Xanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylophilus*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*,

*Arthrobacter*, *Azotobacter*, *Leuconostoc*, and *Alcaligenes*; fungi, particularly yeast, e.g., genera *Saccharomyces*, *Cryptococcus*, *Kluyveromyces*, *Sporobolomyces*, *Rhodotorula*, and *Aureobasidium*. Of particular interest are the pigmented microorganisms.

[00080] Methods of the subject invention also include the transformation of plants or plant tissue with genes which encode the RNAi molecules of the present invention. In one embodiment, the transformed plant or plant tissue expresses antisense RNA and/or RNAi. Transformation of cells can be made by those skilled in the art using standard techniques. Materials necessary for these transformations are disclosed herein or are otherwise readily available to the skilled artisan.

[00081] Additional methods and formulations for control of pests. Control of nematode pests using the RNAi molecules of the instant invention can be accomplished by a variety of additional methods that would be apparent to those skilled in the art having the benefit of the subject disclosure. A "cocktail" of two or more RNAi molecules can be used to disrupt one or more of the genes identified herein. The "cocktail" of RNAi molecules may be specific to segments of a single gene or the entire gene. A "multigene cocktail" of RNAi molecules specific to two or more genes (or segments thereof) is also encompassed by the instant invention. In another embodiment of the instant invention, the disclosed RNAi molecules, cocktails, and/or multigene cocktails thereof, may be used in conjunction with other known nematode control agents and methodologies. Such cocktails can be used to combat the development of resistance by nematodes to a certain inhibitor or inhibitors.

[00082] Compositions of the subject invention which comprise RNAi molecules and carriers can be applied, themselves, directly or indirectly, to locations frequented by, or expected to be frequented by, nematodes. Microbial hosts which were transformed with polynucleotides that encode RNAi molecules, express said RNAi molecules, and which colonize roots (e.g., *Pseudomonas*, *Bacillus*, and other genera) can be applied to the sites of the pest, where they will proliferate and be ingested. The result is control of the pest. Thus, methods of the subject invention include, for example, the application of recombinant microbes to the pests (or their locations). The recombinant microbes may also be transformed with more than one RNAi molecule thereby delivering a "cocktail" of RNAi molecules to the nematode pests. A carrier may be any substance suitable for



delivering the RNAi molecules to the nematode. Acceptable carriers are well known in the art and also are commercially available. For example, such acceptable carriers are described in E.W. Martin's *Remington's Pharmaceutical Science*, Mack Publishing Company, Easton, PA.

[00083] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety to the extent they are not inconsistent with the explicit teachings of this specification.

[00084] Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

#### Example 1—Production of Hairy Roots for RNAi Testing

[00085] A hairy root assay system was developed for testing the anti-nematode activity of RNAi molecules.

[00086] *Agrobacterium rhizogenes*: Several *Agrobacterium rhizogenes* strains produce hairy roots on a variety of plant species. *A. rhizogenes* strains, A4, 15834, 8196 and LBA4404 demonstrate hairy root development on tomato and sugar beet, with A4 being the most efficient. The *A. rhizogenes* strain K599 demonstrated very efficient formation on transgenic soybean hairy roots and was also effective on sugar beet and *Arabidopsis*. However, strain K599 failed to produce hairy roots on tomato tissues possibly due to hyper-virulence.

[00087] Hairy root production: Transgenic hairy roots were identified by stable GUS expression in tomato, sugar beet, soybean and *Arabidopsis*. The construct pAKK1401 (pNOS / NPT-II / tNOS // pSU / GUS / tNOS) was used to produce hairy roots when transformed into *A. rhizogenes* strains A4 or K599. Transgenic roots were identified by GUS expression.

#### Example 2—Protocol for Electro-competent *Agrobacterium* and Electroporation

[00088] Electro-competent *Agrobacterium* Protocol:

- [00089] 1. Grow *Agrobacterium* overnight in 5 mls LB + antibiotics at 30°C on shaker (for *Agrobacterium rhizogenes* strain K599 no antibiotics are needed).
- [00090] 2. Use the 5 mls of overnight culture to inoculate 500 mls LB + antibiotics at 30°C on shaker. Grow overnight.
- [00091] 3. Add liquid culture in eight 50 ml polypropylene orange cap tubes.
- [00092] 4. Centrifuge 10 min., 4000 rpm, 4°C.
- [00093] 5. Resuspend cells in each tube with 20 mls 10% glycerol (on ice)
- [00094] 6. Centrifuge 10 min., 4000 rpm, 4°C.
- [00095] 7. Resuspend cells in each tube with 10 mls 10% glycerol (on ice).
- [00096] 8. Centrifuge 10 min., 4000 rpm, 4°C.
- [00097] 9. Resuspend cells in each tube with 2 mls 10% glycerol (on ice).
- [00098] 10. Aliquot 50 µl into cold Eppendorf tube and place onto dry ice.
- [00099] 11. Store electro-competent cells at -80°C. These cells can be used for up to two years.

[000100] Electroporations:

- [000101] 1. Add 1 µl to 5 µl of DNA (resuspended in H<sub>2</sub>O and not TE or other buffer) to 50 µl of *Agrobacterium* electrocompetent cells and mix.
- [000102] 2. Transfer 20 µl of DNA/*Agrobacterium* mix to cuvette.
- [000103] 3. Electroporate:  
25µF, 400 Ω resistance, 2.5 volts (0.2cm cuvette) or 1.8 volts (0.1cm cuvette for BioRad electroporator. 330 µF, 4000 kΩ, low w, fast charge rate for BRL Electroporator.
- [000104] 4. Add 1ml of LB and transfer to Eppendorf tube.
- [000105] 5. Shake at 30°C for 2 hours.
- [000106] 6. Centrifuge down cells (2 min. 14 krpm).
- [000107] 7. Plate all onto LB + antibiotics (most *Agrobacterium* strains are naturally streptomycin resistant).

Example 3 – Protocol for Production of Transgenic Hairy Roots on Soybean

[000108] Seed Sterilization. Rinse the soybean seed with 70% ETOH for 2-5 min. Remove and add 20% Clorox and shake for 20-25 min. Rinse 3X with sterile water. Plate the seed, 5 seed per plate, onto ½ MSB5 + 2% sucrose + 0.2% gel (referred to as ½ MSB5). Place seed into chamber at 25°C, 16/8 photoperiod for 5-7 day (depending on genotype) germination period. After 1 week seedlings can be placed into cold room for longer storage if necessary (not to exceed 2 weeks).

[000109] Agrobacterium Preparation. For *Agrobacterium rhizogenes* strain K599, take a small sample from frozen glycerol into 25-50 ml of NZYM media with 50 mg/L kanamycin in a 125-250 ml Erlenmeyer flask. Place onto shaker at 28-30 °C for 16 - 20 hours. Pour sample into centrifuge tube and centrifuge the bacterium at 4000 rpm for 10 min. Pour off supernatant and re-suspend the pellet with an equal volume of liquid ½ MSB5 + 200 µM acetosyringone. Use pipette to re-suspend the pellet and homogenize the sample (remove all clumps). To determine O.D., prepare a 1:10 dilution by putting 900 µl ½ MSB5 into cuvette and add 100 µl of bacterial sample. Determine the O.D.<sub>660</sub> and calculate the volume needed to adjust (dilute) OD to approximately 0.2 for inoculation. Check final O.D.

[000110] Explant Preparation and inoculation. Place a sterile filter paper onto plates of 1/2 MSB5. Cut soybean cotyledons just above the shoot apex and place onto plate. Lightly scar the cotyledon's abaxial surface (flat side, upper surface that reaches toward sun) with a scalpel blade. Cut each cotyledon transversely into 2-3 pieces (no smaller than 1 cm). Add approximately 10 ml of prepared bacterial solution to each plate and allow cotyledons to incubate for 1 hr. Remove the bacteria using a vacuum aspirator fitted with sterile pipette tip, ensure that there is no standing liquid. Orient all explants with abaxial surface up and wrap plates for a 3 day co-culture, 25°C in light (16/8 photoperiod).

[000111] Hairy root selection and maintenance. After 3 day co-culture, wash explants with liquid ½ MSB5 + 500 mg/L carbenicillin. Transfer the explants abaxial side up to selection media, ½ MSB5 supplemented with 500 mg/L carbenicillin and 200 mg/L kanamycin. Roots should develop in approximately 2-3 weeks. The roots will form primarily from the cut vascular bundles with other roots developing from the small cuts on cotyledon surface. Remove roots (>1cm in length) and place onto replica media with

transfers to fresh media every 2 weeks to prevent *Agrobacterium* overgrowth. After 6-8 weeks on selection the roots can be moved to media without kanamycin, however carbenicillin must remain in media for several months for continued suppression of *Agrobacterium*. At this stage roots can be used for testing RNAi for nematode control. Sterilized nematodes can be added and observed for RNAi affects.

Example 4 – Testing of RNAi for Plant Parasitic Nematode Control.

[000112] Various types of nematodes can be used in appropriate bioassays. For example, *Caenorhabditis elegans*, a bacterial feeding nematode, and plant parasitic nematodes can be used for bioassay purposes. Examples of plant parasitic nematodes include a migratory endo-parasite, *Pratylenchus scribneri* (lesion), and two sedentary endo-parasites, *Meloidogyne javanica* (root-knot) and *Heterodera schachtii* (cyst).

[000113] *C. elegans*: RNAi vectors can be tested through expression of the RNAi in *E. coli*. *C. elegans* are fed *E. coli* and assayed for their growth by measuring growth of nematodes, production of eggs and viability of offspring. Another approach is to inject dsRNA directly into living nematodes. Finally, soaking nematodes in a solution of *in vitro*-prepared RNAi can quickly establish efficacy of treatment.

[000114] *P. scribneri*: The *P. scribneri in vitro* feeding assay uses a corn root exudate (CRE) as a feeding stimulus and both the red dye Amaranth or potassium, arsenate as feeding indicators. Feeding is confirmed after seven days by the presence of red stained intestinal cells in live worms exposed to the Amaranth or death of worms exposed to arsenate. This bioassay is used to test soluble toxins or RNAi. *P. scribneri* has also been cultured on wild type roots of corn, rice and *Arabidopsis*, and on A. rhizogenes-induced hairy roots of sugar beet and tomato. *P. scribneri* is very valuable in evaluating transgenic hairy roots because of the non-specific feeding of these worms.

[000115] *M. javanica*: Nematode eggs are sterilized using bleach and are used to inoculate hairy roots expressing RNAi. Nematodes are assessed for their growth by measuring knots, egg masses or production of viable eggs. An alternative approach is to microinject dsRNA directly into root feeding sites or into living female nematodes.

[000116] *H. schachtii*: Cultures of this nematode were maintained on sugar beets. Nematodes eggs are sterilized using bleach and used to inoculate hairy roots

expressing RNAi. Nematodes can be assessed for their growth by measuring knots, egg masses or production of viable eggs.

#### Example 5 – Plant Expression Vectors for RNAi

[000117] Modular Binary Construct System (MBCS): An important aspect of the subject disclosure is the Modular Binary Construct System. The MBCS eases the burden of construct development by creating modular pieces of DNA that can be easily added, removed, or replaced with the use of low frequency cutting restriction enzymes (8-base cutters). These constructs are useful for delivery of a variety of genes to plant cells and is not limited to the delivery of RNAi genes. To develop this system, a series of six, 8-base cutter restriction enzyme sites was placed between the left and right Ti borders of a previously created *kan<sup>R</sup>/tet<sup>R</sup>* binary plasmid (Figure 1). The production of both *kan<sup>R</sup>* and *tet<sup>R</sup>* MCBS aids the testing of constructs using different strains of *Agrobacterium rhizogenes* in different plant species. In addition to the MBCS, a series of shuttle vectors were created that aid in the cloning of useful DNA fragments by containing the multi-cloning site (MCS) of a modified Bluescript plasmid flanked by 8-base restriction sites (Figure 2). With six 8-base cutter sites, each site is, preferably, reserved for a particular function (Figures 3 and 4). Because of the close proximity of the *Pme* I and *Sgf* I sites to the left and right border of the binary vector, these sites are, preferably, reserved for gene tagging and enhancer trap experiments. The *Not* I site is, preferably, reserved for plant selectable markers (Figure 5). The *Pac* I site is reserved, preferably, for Plant Scorable Markers (Figure 6). The *Asc* I site is, preferably, reserved for RNAi experiments (Figures 7 and 8), while the *Sbf* I site is, preferably, reserved for anti-nematode proteins. The restriction sites that are denoted in the Figures are, preferably, reserved for the denoted insertions; however, the MCBS binary and shuttle vectors do not require the restriction sites to contain these suggested inserts.

[000118] Plant Selectable Markers for MBCS: To further develop the MBCS, a series of plant selectable markers were added to the MBCS (Figure 5). Plant selectable markers that were added to the MBCS include: *pNOS/NPT-II/tNOS (kan<sup>R</sup>)*, *pNOS/Bar/tNOS (basta<sup>R</sup> for dicots)*, *pUBI/Intron-Bar/tNOS (basta<sup>R</sup> for monocots)*, and *pUBI/Intron-PMI/tNOS (mannitol isomerase<sup>R</sup>)*.

[000119] Reporter Genes for MBCS: Four exemplary reporter genes are used in the MBCS are provided in Figure 6 and Appendix 2. GUS, a nuclear localized GUS, GEP, and the anthocyanin transcriptional activator *papIC* genes into the MBCS.

[000120] Promoters for MBCS: We cloned several useful constitutive and nematode-inducible promoters (Figures 6, 7 and Appendix 2). Constitutive promoters include the SuperUbiquitin promoter from pine (pSU) and two promoter regions from the Strawberry Banding Vein virus (pSBV<sub>1</sub> and pSBV<sub>2</sub>). Seven nematode-inducible promoters from *Arabidopsis* were also been cloned.

[000121] The following Scorable marker clones have been constructed and placed in the MBCS, NPT-II binary vector (pNOS/NPT-II/tNOS):

Intron/GUS/tNOS	Intron/NLS-GUS/tNOS	Intron/GFP/tNOS
pSU/Intron/GUS/tNOS	pSU/Intron/NLS-GUS/tNOS	pSU/Intron/GFP/tNOS
pSBV <sub>1</sub> /Intron/GUS/tNOS	pSBV <sub>1</sub> /Intron/NLS-GUS/tNOS	pSBV <sub>1</sub> /Intron/GFP/tNOS
pSBV <sub>2</sub> /Intron/GUS/tNOS	pSBV <sub>2</sub> /Intron/NLS-GUS/tNOS	pSBV <sub>2</sub> /Intron/GFP/tNOS
pKT/Intron/GFP/tNOS		
pKA/Intron/GFP/tNOS		

#### Example 6 – Control of Plant parasitic nematodes using RNAi in planta

[000122] Production of RNAi Vector. The RNAi shuttle vector to be used is adapted from the Modular Binary Construct System (MBCS - See Example 5). RNAi shuttle vectors preferably comprise a promoter, intron, antisense RNAi, stuffer fragment, sense RNAi, and terminator (See Figures 7 and 8 and Appendix 2 for more details). The plant promoter can be constitutive, tissue-specific or nematode-inducible. The intron is necessary to eliminate expression in *Agrobacterium*.

[000123] The anti-sense and sense RNAi molecules comprise nematode-specific sequences and are disclosed herein. These genes are associated with pathogenesis, growth, or other cellular function in nematodes. An exemplary group of RNAi sequences for use in plant/nematode control may be based upon:

[000124] 1. Genes specific for nematode esophageal gland cells.

[000125] 2. Genes specific for plant parasitic nematodes but not other free living nematodes.

- [000126] 3. Genes common to all plant parasitic nematodes.
- [000127] 4. Genes common to all nematodes (nematode-specific).
- [000128] 5. Genes specific for important tissues or cell types.
- [000129] 6. Genes from large gene families.
- [000130] 7. Genes involved in nematode signal transduction or other cellular pathways.

[000131] Appropriate RNAi constructs allow for the formation of dsRNA molecules (the sense and antisense strands join to form the dsRNA). The terminator sequence adds a poly-A tail for transcriptional termination. The RNAi shuttle vector can then be subcloned into the MBCS and transformed into *Agrobacterium rhizogenes*.

[000132] Plant Transformation with RNAi Vectors. An exemplary transformation system for generating hairy roots using *Agrobacterium rhizogenes* is provided below. The RNAi vector once introduced into the MBCS can subsequently (as a binary vector) be transformed in *A. rhizogenes* using, for example, the electroporation protocol of Example 2. Once the *A. rhizogenes* is confirmed to contain the plasmid, it is then used in generating hairy roots (See Example 3). Using this protocol transgenic hairy roots expressing RNAi are isolated, cultured and tested.

[000133] Testing of RNAi Vector for Nematode or Plant Pathogen Resistance. RNAi expressing hairy roots can be inoculated with sterilized nematodes. Infested hairy roots can be observed and the effect on nematodes determined. An alternative approach involves the microinjection of RNAi directly into root feeding sites (giant-cells for root-knot nematode, and syncytia for cyst nematodes) or into living female nematodes.

#### Example 7 – Insertion of Genes Into Plants

[000134] One aspect of the subject invention is the transformation of plants with genes encoding proteins of the present invention. Transformation of plants as described herein can be used to improve the resistance of these plants to attack by the target pest.

[000135] Genes, polynucleotides, and/or RNAi molecules as disclosed or suggested herein can be inserted into plant cells using a variety of techniques which are

well known in the art. For example, a large number of cloning vectors, for example, pBR322, pUC series, M13mp series, pACYC184, pMON, *etc.*, are available for preparation for the insertion of foreign genes into higher plants via injection, biolistics (microparticle bombardment), *Agrobacterium tumefaciens*, or *Agrobacterium rhizogenes*-mediated transformation, or electroporation as well as other possible methods. Once the inserted DNA has been integrated into the genome, the genetically modified-cell(s) can be screened via a vector carried-selectable marker that confers on the transformed plant cells resistance to a biocide or an antibiotic, such as kanamycin, G418, bleomycin, hygromycin, chloramphenicol, or bialaphos, *inter alia*. The transformed cell will be regenerated into a morphologically normal plant. The transgene(s) in the transgenic plant is relatively stable and can be inherited by progeny plants.

[000136] If a transformation event involves a germ line cell, then the inserted DNA an corresponding phenotypic trait(s) will be transmitted to progeny plants. Such plants can be grown in the normal manner and crossed with plants that have the same transformed hereditary factors or other hereditary factors. The resulting hybrid individuals have the corresponding phenotypic properties.

[000137] It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.



We claim:

1. An RNAi molecule, optionally comprising a linker, wherein at least one strand of said RNAi is encoded by a DNA sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 139.

2. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
1.

3. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
2.

4. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
3.

5. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
4.

6. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
5.

7. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
6.

8. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
7.

9. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
8.

10. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
9.

10. 11. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
12.
11. 12. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
11.
12. 13. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
12.
13. 14. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
13.
14. 15. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
14.
15. 16. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
15.
16. 17. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
16.
17. 18. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
17.
18. 19. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
18.
19. 20. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
19.
20. 21. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
20.

21. 22. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 21.
22. 23. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 22.
23. 24. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 23.
24. 25. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 24.
25. 26. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 25.
26. 27. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 26.
27. 28. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 27.
28. 29. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 28.
29. 30. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 29.
30. 31. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 30.
31. 32. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 31.

32. 33. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
33. 34. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
34. 35. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
35. 36. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
36. 37. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
37. 38. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
38. 39. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
39. 40. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
40. 41. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
41. 42. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
42. 43. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

43. 44. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
44. 45. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
45. 46. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
46. 47. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
47. 48. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
48. 49. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
49. 50. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
50. 51. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
51. 52. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
52. 53. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
53. 54. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

55. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
54.
56. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
55.
57. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
56.
58. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
57.
59. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
58.
60. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
59.
61. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
60.
62. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
61.
63. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
62.
64. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
63.
65. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
64.

66. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
65.
67. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
66.
68. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
67.
69. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
68.
70. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
69.
71. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
70.
72. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
71.
73. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
72.
74. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
73.
75. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
74.
76. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
75.

77. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
76.
78. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
77.
79. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
78.
80. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
79.
81. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
80.
82. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
81.
83. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
82.
84. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
83.
85. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
84.
86. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
85.
87. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:  
86.



87. 88. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
88. 89. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
89. 90. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
90. 91. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
91. 92. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
92. 93. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
93. 94. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
94. 95. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
95. 96. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
96. 97. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:
97. 98. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO:

99. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 98.

100. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 99.

101. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 100.

102. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 101.

103. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 102.

104. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 103.

105. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 104.

106. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 105.

107. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 106.

108. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 107.

109. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 108.

110. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 109.

111. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 110.

112. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 111.

113. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 112.

114. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 113.

115. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 114.

116. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 115.

117. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 116.

118. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 117.

119. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 118.

120. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 119.

121. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 120.

122. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 121.

123. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 122.

124. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 123.

125. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 124.

126. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 125.

127. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 126.

128. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 127.

129. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 128.

130. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 129.

131. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 130.

132. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 131.

133. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 132.

134. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 133.

135. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 134.

136. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 135.

137. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 136.

138. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 137.

139. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 138.

140. An RNAi molecule according to claim 1, wherein said DNA sequence is SEQ ID NO: 139.

141. A transgenic plant or transgenic plant tissue comprising an RNAi molecule according to any of the preceding claims.

142. A method of disrupting cellular processes in a nematode comprising the steps of:
- (a) providing a composition comprising a compound according to any of the preceding claims; and
  - (b) contacting a nematode with said composition.

143. An isolated promoter comprising the following nucleotide sequence:

aacagcccaagataaca gaaaagtcaaaggtgttcgaaa  
gaccacttgtgactaaggatcattt catccataattatctggtagca  
cagactcatgataaetgcgaggaacacaagtctcttacagtcgattc  
aaagacactttctctttacggtttcattgaaggagccgacccagaat  
atgtcagagaagcttttctactgtgggttaatttcattaatctatcca  
ggtgaaaacctcaaggagatctctcttctccaaaagacctctacag  
ggcaatcaaaaactacagaaccagagtttgtagtgcacagagtagac  
caatctacctgagaatcacgagtaccttcttagagtgggaaaatgat  
gacatccttattccataccactgga ttgaggtaggactatccaatgg  
aaaaattccatgggacaagtcatat aagaagaccgcaacagtcgagt  
atcttccagagataactgcactcagacctaaggataaaaagcagta  
tataatcagtggtactaagatcttcgcagattcaaagaagaagcttaa  
ctatgctgatgacaagataattctaataagcaattattcagaattaa  
tcaaggagaaagaattaataactcttctcagaatatgaagcccgcttt  
acaagtggccagctagctatcactgaaaagacagcaagacaatggtg  
tctcgatgcaccagaaccacatctt tgcagcagatgtgaagcagcca  
gagtgggtccacaagacgcactcagaaaaggcatcttctaccgacaca  
gaaaaagacaaccacagctcatcatccaacatgtagactgtcgttat  
gcgtcggtgaagataagactgacctcaggccagcactaaagaagaa  
ataatgcaagtgggtcctagctccactttagctttaataattatgttt  
cattattattctctgcttttgctctctatataaagagcttgatattt  
catttgaaggcagaggcgaacacacacacagaacctccctgcttaca  
aaccatgtattgtagctaaacctcttaggag .

144. An isolated promoter comprising the following nucleotide sequence:

tggtggggacaatggatccggtctgcgtagcaacaaggctg  
aaaaagattaaacagaaacctgtgatcattagcgttgaccaccacc  
aaaacctcctgagccaccaaaagcctccagagcctgaaaaaccaaagc  
ctccaccagcacctgaaccaccaaaagcatgtatgcaagccaccttac  
tgcaacagttgtgatgttgtgtctgttactacctatgaaagtggaag  
cggctgcaccattctttgagtcataatatcgcgtagcatagccttcac  
gttaagtcctgtatttagccaataactaatcatcatgttctcatgct  
tttttggtttatttctttttctcaaatatgaatctctgtttgttcc  
ctccctgtttataattagtcgcttctttgacacaagaagtctcatg  
agttcatgctaaagaaaataaaaagttcaaattaaaacaccaaagtgt  
tgattaatttccataaacctgtgaagcagaaagttagtcattgtgac  
ctgaacagagcttaggaagtccttgaaggacatatcttcaagtgtata  
ttgggtcgtagcactcttaggccatttaacttcattgagccattaa  
attatgcaaaacaagaatgagacatatggaacattagggttctta  
caggaaaaaataggaaaaagcaggggacaactaaacaaaaattcagaa  
acaagaggcaagtggacgaccacggcgtaagatcaacatgtggtgat  
gtgcatgagaccaagaccattttttctcgttcttcaacgcacacttg  
gtcttttcttatgtttgttgcatctctttatttaggcagaccctctct  
cttttttaataggatagtaaaaaatatatgattttattttgttgaaa  
cattttgagttaaaacctaaacttatagtaagcattttagtagtgaa  
tttccctatagcatctatcaacatgacctctaacaaaaaaatatt  
gatgaaactactttaagtagtaaaacctaaagcaattaaaatttctt  
ttaaattagtagtttgtgttaaatttaattgacatgattgcgtcgaaag  
aatcaaaacagttatatcgtgaacttaggagaatgttttatatcgt  
gtttcaacacatgattgctagcatatgtgtaggtgtcgtagacgtta  
cataacaatcatcactcgtaaatatcaaagtggtttctgagagaaac  
aaaggggtatgattttcccaactgcactagttgtgtattgtttcttt  
cacacgtatgcttctgagttctgcccagtggaattaaagcagag  
ttgggagagatcataatttattaggggtcgttatgctcaagtcatga  
cgtaaaatgaaaatttggtttttattctttcaccaacacaaagaatag  
ctagttatctctttttttatatataacaattcatgaagttgatcagc  
tttatacacatcatccaatcgaaattgctaattctagagatggaaatat  
caggatagagccaataagatatcaaatccaatggaccattttctcc  
atgtgctaattcatacaatctgtttttgtctgctttatttgatgatg  
atgctgagcgttttttaagtgtgaactaagatctagctaaccaaaaca  
aagatgggtctcttctgtctttgtcgtataagagcaagagagtgggtt  
gattcaatttttaaaattctaaataaaaactccaaccgtgaatccagc  
catgaaactcttttagaaaatcctttttataacaaataattctct  
tgcttcttcttcttcttctgtttatttcaacctttttgggttcttttag  
ctcagaaaaagcccatcttttttctattcttgtttattttaatca  
tactgtgcgtttctacaaagtttggttcttcttcttcaactctctc  
actcacagtcacagagatctgtttcttttcttttttgcttctactc  
ttctcttccagt.

145. An isolated promoter comprising the following nucleotide sequence:

.agcaaagcaagaacaccagagaagaagaaaagcactacaga  
gaaaaatgtgagcttaagcgctctccaacaacacttctctgggagtc  
taaaggatgctgcaaaaagccttgggtggtagacttccgcatatttc  
caagcatgggtttatTTTTgttagcacacaaactatctgaccctega  
cttggattttctctctgcagtttgtccaactacattgaaacggatatg  
caggcaacatgggatcatgaggtggccatctcgtaagattaacaaag  
tgaacaggtcactaaggaaaatacagacggtagctggactcgggtccaa  
gggtgtagaaggaggactaaagttcgactcagcaactggcgaattcat  
tgcagtttagaccttttattcaagaattgataccctaaagggtctgt  
cgtctcttgataatgatgcacatgcaagaagaagtcaggaggatag  
cctgacgatacttcattcaagctccaggaagctaaatctgtcgacaa  
tgccattaagttagaggaggatacaaccatgaatcaagcaagaccag  
gtaagaacttctctatccataaacctagatggagcgattagaatct  
  
taatccattttcagtttttgcaggatcattcatggagggttaatgcta  
gtggtcagccatgggtctggatggccaaagagtcctgggttgaatggc  
agtgaaggaataaagagcggttgcaacttaagctctgtggaaatttc  
agatggaatggatccaacaatccgatgcagtggcagttattgttgaac  
ctaaccaatccatgtcatgcagcatatcagattcatcaaatgggtca  
ggcgagttctgcgtggaagctcatctacttccatggaagattggaa  
ccaatgagaacccacaacagtaatagcagcgagagtggtcaacaa  
cgctgatcgtaaaaggccagttatagagaagacactgtacgtttcaag  
ttcgagccatcagttgggtgtcctcagctctacaaagaagttggaaa  
acgttttaactgcaggacgggtcgtttcagctgaagtacttggatg  
atgaagaagaatgggtgatgctgggttacagattctgatctccaagaa  
tgtttggagatatcatgggtatgggaaaacactcgggtgaagtttct  
cgttcgtagtttgtctgcccctctaggtagttctggtggcagtaatg  
gttatcttggaacaggcttatgacgtcgttaagacatagacacacaca  
gttatgtattcccagtgaaagaattgttgtttatttctctagatatta  
gtatgcttataaataggcatgaaggagaaagacaattttggtatagt  
ggagttcagcagaaaaatgtatatgtttttcgttttatatgaatcag  
agaataaaaagttggatgttatatctacgttgctaattgtgtacctgc  
tcacccatctttcatataagaaaagagaacacttttagttatccctg  
tgatgcagaatcgattctttgttatctctccattcctgtggaaacc  
aacaagtcactaaatttcgggttaattgggtgggttttaagtcaa  
cgaggacttgatttttagttgggttgggcctataattgtgttcatca  
ttgggttttttcccccttatcagtttaacgtccatatccatatcttt  
ttcttttttaacggcaaagttcatatccatatcttatgatgtgcct  
aaaagaggggagaagatgcgaagacagaattttcatatttgaaagggt  
tcgatatcgatattgggaaacgaattcaagggtcaaaaaactcagtcta  
atagttgaaatttaaaaattttatttaattcaatccgattgggttcgt  
ttgttatgggtcggttctatatcatcaaaccaatcggtttgggtcct  
aaagataattataaatattaccaaacaccagtggttaaacacatatca  
acaaacctaaagttagataaacaagaga.



146. An isolated promoter comprising the following nucleotide sequence:

aattggcactcttcttctctgctgggttccaaaagaaacgaat  
caatatgtgcaacaagaagagctccagaagcagtcattcttctaaaat  
cttaatctaacaacagctcaagaagaaaaaattccatagctagaga  
gaacacaaagtcaacaagacgacgctcgtagaggcacaagtcacacct  
gaatggcttaagccgaactgagtggttttgactagaccatcatcaga  
aaagtcctccaagacggtagtcggatgttagatcgctcaagtaatttt  
tgggttttggttggtctcacgtcttcagctgcccatttgatttcagttt  
gggcttttcccttatctctaaaaggcccaatttcatttaggttagttt  
atttgatcattatccttactataaaggcttcgcctttcgagaaattt  
agggtttctctctgtctgtctcgtcactcagggttgtgcctcaacgac  
tgcttcacttctagcttgattcttcttcttcgtttatatgtatactg  
tacattagattattcttggttctcagagcttctgctatagattttgat  
tcttttttttggttgtcttggttcgtttccaggatcagatcttagct  
aaattgagacaagctcaaaatgaggtacttgacgcattcttcttaoatt  
cactgtttaattagagaacaaataggtctctgaatcgtgattcagaga  
cgtattgttcttctgtcatatgcaataagttaattagagaacaata  
cgtctctgaatcgtgattgttctttggatgtgcgttattgatagctt  
tatgatgttaatagcttaggaatgacacgaagtgttctgcagtttt  
gcataaatgctctttactaaaggcctctaaatttggtatgacaaatcta  
aatcttgccctcataaaaaatttaggtgtattaagataagattatttg  
tatggtagtgctataatgtgggttgttcattgtgaggtgtcaatg  
ttgtgtatttttggttgttttagttaatttgcttaactctgttctttg  
tgggttaatacagtaagcttcagagtgaggccgttcgtgaagccatc  
actactatcacagggaatccgaggcaagaaacgtaactttgtcga  
gactattgagctccagatcggctctgaagaactatgaccctcaaaagg  
acaagcgtttcagtggtatctgtcaagttaccacatatccccgctcct  
aaaatgaagatctgcattgctcggagatgccagcatgttgaagaggt  
gatatatcttttcatggaaatcgatcattttgtgctctgtttcttgt  
ataatggttttgtgctcatttcatttggtggtctctattagtttcatt  
tgatgttgatatagtcttctgaatgtagatgcattgatttttcggaa  
tttggtcattgtttatttaggcttcatttcttgcataattaaatatt  
tgcttatttcattctgtatctcttcgtaggctgagaagatggggttg  
gaaaacatggatgttgagtcctcaaaaaagcttaacaagaacaagaa  
actcgtcaagaagcttgcaaaagaaataccatgctttcttggcctctg  
agtctgtcattaagcagattcctcgtcttcttgggtcctgggtcttaac  
aaggcaggcaagtctcggctaagcctaataatccattgttcttcttt  
acatccgttttgattttggatagggttttagtagtctatttcttttgg  
caatgtctttttgatacaatgccaatcctttatcctgtgagattatg  
cttctttgatgattcttaagtacattcctttgctttactttacaca  
ggaaaattcccaactcttgtgagccaccaggaatccttggagtcaaa  
ggtgaatgaaacaaaggcaacagtgaaagttccagctgaagaaggttc  
tgtgcatgggagttgcagttggttaacctt.

147. An isolated promoter comprising the following nucleotide sequence:

ttggcaaaactgagatat aagaggggaaggtgattttcatgcaa  
attttttttttttttttttttgaatgaatgcaaaatttattcaaaaa  
aaaaaaacctgggctacatcaagtacttcatttctgagtttttga  
aatctaaagacaacaaaagactttacaatttaataaaaaataata  
aaatactttatcactctcaacgaaattgttgatttaataacgtatct  
cttgggtaaaacagcggttttatttgacgaaattgttataaatgaata  
aatgataatagaaactagtgtgggtacgtaaaatacctctcatttggc  
aaaataacggttatgtatcatgagatttgcatacgacagcggtgctta  
aatagtgtgctttcaggagaaaaatatataccaagttatttgcgtgaaa  
ttaccacgcaaatctgaggttcgaatggcaaaaataaaaaaccaatgt  
catttccctaatgtatttaaggtcattttaaataaaaattgtacactttt  
ttcacctgtaagcggtcccaaagtgttagaatggataactagaaggggtc  
aaaggtataatattaataagcgaactcactttttgcccagtgattt  
cacttcttacatttgccttgataatagttacccaaaagtgtatatatat  
tcccttatacaattgttctattttctggattataaggggaataagaa  
aaaagaaaagagagagtataataataactttttataaagtgatgtta  
gattctaatttgtaacgaaaagt tcaaagtgaagaaaaaacgaaaa  
agtttttctgttttgttttatatctatagccaagaaagtttctcaga  
tttacaagaagttaactgagaaaaacaaaaaaaacttatgaagca  
tgaaagactaattaacgaggtgatttaattttgagacaaattaaacat  
cgaattaaaagtaacatttggaggggtttatatgttatatatgtgaca  
tgataagtccgattcatgactaatgtatatctggaatctaactgga  
agaatagagaacgaagcagagcccaaggtcaacttgccagacacgaat  
caacagattgtgaatgagaccaaatacaatgggtcataaacgggtggg  
tttaaacgggcaagtcactcttgggtcaattccattcggtattcctt  
catgcaagaccctctgatacaaccaaaagactcccattacaatatctt  
ttcgatcacgagctacttattttcaaagtgttacctctttcgtgac  
tcttgtgttgtgtggttaaagcctagtcgagatgtgtcggtatatata  
ggcatacatatacaaatgcgacaaaataagttatattatattgtttaa  
tttctatattccatttctatatgcatgggtgggatttttgacaaaa  
ccctaattcaagaatagaatccaaaagatgggatcaaagaatataat  
ctaattgggctgaccacattttccgatttaattcgcatagttaatatt  
ctttccactactttatgccgcagaaatttgtaatttaagtaagacaaa  
gaaatacagatataagatgggtcgtagaaaccagtagaggaatttcat  
tttctgtggataagtggaatatt aataagagaatgggtctttactctt  
tacagtgggaaatgggaatagtagccattataatttcatcagattc  
tatatatgcatgtttgtataagctaaaataaaatacgtttaagcattc  
ttcaaaaaaatttacaagttctagagactctcttaacgtcggcaatt  
tatattctactttacatgacactttcaggaaaagaaaactataactca  
ctagcagatcattaaattttctt tttctttttttgaatgaaccttag  
ttgtgggtttttatttttgttagctagaaacttcagtgtttttttcc  
gccaatggtagtgctttgatgatgggtccgg .

148. An isolated promoter comprising the following nucleotide sequence:

caatcaaggtaacgaaggaggatcagcgaaaggatgggcta  
tat t t g g a g t t t t t c c t g c g t g t a a g t a a t g c t t t g t g a t c t t c c a  
t g c g g a c a t a t a a c t g a a g a a t a a a c t c a a c t c a t t g t g t t c t g g t g  
t g t t t c t t c t g a t c a g a t t c c t c g t t g c a t c t g c a c t t t t c t g c t g t  
g g g g g c t t t a t t t a t a a a c a a g a g t a g a g c g t g t g g t a a t c t t c a t  
a t c t t t c t a c a a t t c c a c t t c c a t t c t c t a a t t a t t c t c t c a c g t g a  
t a t a c a c a c a c t c a a t c a c t g a t g t a c t c g t a t g g a t g c a g c g t g g a  
a c t g a t g c a t t g c c g g g a t g t c a c t t c t a t c g g g c t t a c t a g a a a c  
t g t a a g t a t t a c a g a a a a c t c a a a a g g a t t c c a t t t a t g c a a a a t c  
t a a g a g a a a g c t c a c t g t g g t c t t t g g t t a c a a t t t a t g g a t c t c t c  
a a g a g a c a a a t g c t a t g t a a g c t a a t t g a t t t t g g t c t t g a t a a a c a  
g g t g a g t g g a a g t g g a c a a a g c t a c t c a a g a a c t g a a g a c a t c a a c a  
a t g c t t t t g c c a a t g a a g t c t c a t g g g a c c g c t c t t c c g c a t c t t c t  
a c t c a a g c g a c a c a a c a c a g a g a c c a a g t g a a a g a c a t a t g g t g c  
g a t c t a a t t t t g t c a a g t g c c t c a c a a g a g g t a c t g t t t c a a g c c a t  
g g t a t g g c a c g c t t g t g a t c t g c g a t t t c t g g a t t t t g c t t t g t a t g  
t t t a t t t t c t a c c t t c t a g a a a g a g g t c a a a a g t t a a t a g c t t c a c  
c g t g a g a a t g t t g t t t t c a c c a g a t t c a t g t g c t a t g a t a g a a a a g  
a c a a a g c a a a c a a g a g t t c t t t c t t t g c t t a g g t t a c a a g a c a a g a  
g t a t c g t t a t a a a g t c a a c a a a g a t t g a a c a t a t t t t g t c a a g g g  
a g t g g t t a g a a t c t c t t c c t a c t c t c t t g c c t t t c t c a c t a a g a c a a  
a a a a a g a c t t g g a c t t t g t c t a a g g t t t t g t g g a t a t t a t t a a c c a  
a g t c c t t t t g c a a a a g t a a t a t t g t t t t t c g c a t t c c t c t t t t a g  
a a t t t a g t t t a a t c t a g g c t t t a t a t t g g t t a t t a c t t t c t t g a a a a  
a t g a t c t g t t t a t t c t a t t c a t a c t t g g t t a c c t c g c t t t t a t c t t  
a c t t c t a c a a a a g g a t t a t c a g t g a a a g t t a g t c t c t t a c t c t c a c c  
t t c c g a a a a t a a a c a a a a a t a t c g a t a c t t c t a g a t c a a a c c a a g t  
t g a t t a a a a c a t c c c t a t t c c c t a c g a t t c t g a t c t t g a g a t a t a t t  
a t c a t g t t a a g a t c t a a a t t g a c a a g a a a c t g a t t t t t c a t t t c t a  
g t a g g a a a a t a a t t a c t a t t a g t g a t c a t g a t t g t c g a c c g t a a g a  
g g t g g t t t a g t t a c t c t c c a t c t t t c t t t g a a g a a g t c a g a a a g t c a  
g a a a t t a t a t c a a a t t a a c a t c a a t a t t g a a c a c a t a t a t c t g t a t  
g g t t t t a t g t t t a g a a a a t t c c a a t a t t t a t a t t c c t a g g g a a a a  
a g a a g c t t a t t c t t c a a a t t a t t g t t a t g a g t c g t t a a a a t a t g g a t  
a a a a a t a t a a a g t c t a a a t a t t a a a a a c t c a g t t t g c t t t g c t t t a  
c c t c t c c a a g t c t c c a a g t c a a a t t a a t t t t a g t t a a t t a a c c a a  
a a a a g g t t t a t t a g t c a a a c t t a g c a t g c a a t g c t g g g t a c c a a a c c  
c a a g c a t t a g t c t c t t t t a a t c t t c t t t t t c t c c a a t a a g t t t t t a c  
a a t t t t t a a t t g t t t g c a t t t c c c t t g a t t a t t t a t c t t c a t c c c a a  
t t t a g c t a a t a c c a a c t c c g t t t c t t a t t c t t c c a a g t c t t t t c t a  
t a a a t a c g t t c t t c t t c c c c t c t t a t t t c a t a t c a c t c a c c a c a a g  
t c t t c t c a t t t c c t c a t .

149. An isolated promoter comprising the following nucleotide sequence:

atgttgtagtgagtgaggaagaagaagagggaacaaagggtatt  
tattttagtagcgagttttgttttctgtgacgcggttttgtctgtgttcaa  
tggtgacgaaacgagtgagagagtggtctgattattaaagaaaaccct  
aattaagtcagaccgcggttataaaaaatagtcaaaaagtaggaaa  
acgcgtgtgtgagtgagacagagacagccattgtttgctttatggg  
cttataagcgagacgtgttaaattgggctttttcctttatggccgaaa  
acaaaagaaacgtcgctgagagattcgaaactctcgcgggcagagcc  
catgtacttagcaggcacacgccttaaccactcggccaaagcgactt  
gttgctatgagttagacaaaaattcattaaaattctctattatgatttc  
tcatagtggtgtgtatattgtggatctactaaaaattcctttgttat  
tattactttattttgtgaattagtttgatataggttaagtacaaagtt  
aactttattatttactcaaaaatttatcagattaactgattttatatt  
gtttcctttgggtatatagacgtactatagtttttagaaaaaccataa  
gattcctttatatttcatagagtgagagatgagatgagatcttggc  
tggagaagaaataagtttccacgaggaggactcttttttttgggtga  
agacgaggaggaggactcttgggtgatccagtctttacgttagacat  
cgaccctacatttatttggccttctctatcaacatggcaggtaaaa  
atcttcattcaaccgaaccaaaccaggtctcttcccaataatattca  
agcaccatcctttgggaaactcatacatactacagtctacactcttt  
cattttctttcaacgctcaacttaacaaatgatatagttagttgtc  
aattatatgttttaattagtgctttcacatcaaattctgggttgata  
tttgatgactattttcggaacatctcaatgtcccgcaaatacaatc  
gtctatcatatataatcccgtacgttgattcttatagatagaataa  
tatggcggtgatctttataatacaacatatagaatcgtgtagatttat  
tttattttatttttatatatcgcataaattgcaaaaatacttatatat  
gtttgttatatatgataccaattttatagttacttaaaaaaagttaa  
gcgataatatatatatatcaactttttataacaaaaaagtataacac  
atggtaaagaaaaataaaaaatgaagacatgggtgtgacacgaaaatgg  
cactaaatatacatatataatagatagctacaatatcccatcataca  
cacttttttaattgactaatacataacttacacacttttttaattga  
ctaattcataactttttatcaattgtcaacatgcaaattcatatttcc  
gttgaactattattcttattttgtttttaaaagaagggttctctggg  
aataaaaaatagatttccaaaatgacgttagagcaaaaaaaaaaaaaag  
gttggtctgggtctggtaaaatgaaaaagcaaagcgtcttgggtatagaa  
aagtaataactgectcctaaatttctctgctcttctaccgaagaatc  
tctccactcttgccctctttcgaaccctaaaccagaagcaccagat  
tttttcaactttttccagagaacaatagaaaacccaacttgtgtctc  
tctagggtttttctttattccttctcatctttggattttcttgggtca  
tcattttggaagcttaccacacagcgaaaaaattataacttccatcg  
attcctgggtctctctctctcgctctctctgcatgtgctaaatcgccg  
gactgatcctcactgtcacctctgtt.

150. An isolated promoter comprising the following nucleotide sequence:

gattaggggtttgagttgtcactggaaagagggttgattgt  
gagtgatgatggagagattatgaaggagtttgtgtgtatttatagag  
gagttaggggtttgagggttgatgagaagtaggttgaagaagttt  
gttgttgcaacttatttagagttacttgttccacaaccacaagtaag  
attgggtcacttctaagttctaactagaaacaacccatgacacatggag  
atttcagctaaccctagtttaa tgtatatgtatttatattttatttaa  
tattataaaaataaaaataaattttcacaaaataaaagaactacaaaaa  
gtgagaaaaataatttgataa acaaatttagaaaaattagtatatcaa  
taaataaatttataatccgatgggttttgccttttggtttggcctttg  
tttgaacttcgatgagtgactatgtatagcgaaaacaattcgggtttg  
tttttgggtttaatttttaaaaaatacaagcgacaatatctgatgagaa  
taggtgaaaagcaataatatcagtttaattggaaatatctactttt  
ttacaactaatattttgttttggtcaaccaacaatatagatttaattaa  
ttatgggttatgagcttttat tttgttgcgacagtatatatatgttaa  
aatagtgatattgcatggcggaaaggtccggaagcaacacatatctcc  
tttttaattttttttttaacaagaataacatgttaattttttttga  
aattaataaagaatacatatttctaatttttgcgtcagatagatgat  
taaagagtgtgtgttttttttaacaacaaggaatacattatacata  
tttcatattttctcgcacattgtttgttttttaaaaaatagattaa  
agagtctacgaagctaagtagctaacgaagacttgaaatgagaagaa  
gacgagaatcttttaattttttttgttgaagcgataatttttgaaaa  
ttaataaatatagattaaaggaaataaacaataacgcagatatcggtaa  
gtcatagaaaaaaagaacaaacacaaacttacataaacatgtttcct  
aatttglaatggagtaaaattccttcttttttttttttttttttgattt  
ggattccaattagtaagaac tcaatgactataaataacctttaacc  
ctctcattattttcttactatcaattgattaaagctctcgttcctaaga  
aagcaatagacgaacaagaacccatcgaagaacacaaatctctcttt  
gaagttgtcgataatgttagtaacacgttacttcgtccaagactttt  
ttgccgttcggtttcttacaaacaaggatttggttaccattacttt  
tgtcgtaactcctttttacatgtacgtcaaaaagtggttcctcgtc  
cggcttgaagaaacgaccttc taccacaaaaagcttattttaaac  
cgtctaaaaccggaaaatctcaatctaaaccggatacggttcatgag  
aaaccgattcaaaccaggagtgaagaagtagaattttttgatgggtc  
cgtcacaaatgtgtgctgctcc ttcgccaagacatgtaccgattccga  
tattttgtggtgtaaagatga tcaaagagtcttcaaagctaagcacg  
acttgaatgagaagaagaaga ccaattactcaattagattttgtttt  
gtggagcaattattgtctatt t atctttgttttttagcaaaataatctg  
tatccactaatcttcacagta cttgactaacaagaagtaagaggttt  
tcttattttccaattgtttttta atctgatacttttttcataatttta  
caatgtttgatgaaaaaaaacattcaaaccctaaattttctttttttg  
gtatgaattcaaaccctgaattacttttgacgaggacccgacgggtata  
aatagggtgatctcccaacaaacaaaaaggggt.

151. A transgenic plant or transgenic plant tissue comprising an isolated promoter according to any of claims 143 through 150.

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APPENDIX 1

SEQ ID NO:	INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTID E / GENE
1, 2, 3	2293133	glyceraldehyde-3-phosphate-dehydrogenase
4, 5, 6, 7	7143495	Histone H4
8 & 9	7143515	ATP dependent RNA helicase, mRNA sequence
10, 11, 12, 13	7143527	nematode specific
14 & 15	7143602	protein serine-threonine phosphatase 1, catalytic subunit
16 & 17	7143612	40S ribosomal protein S4
18	7143666	cytochrome p450
19, 20, 21, 22	7143675	Neuroendocrine protein 7B2
23, 24, 25	7143839	nematode specific
26	7143863	40S ribosomal protein S17
27 & 28	7144016	vacuolar ATP synthase subunit G
29	7144025	malate dehydrogenase
30 & 31	7144060	J2 pcDNAII Globodera rostochiensis cDNA similar to Bystin, mRNA sequence
32 & 33	7144225	similar to arginine kinase
34	7144354	pyrroline-5-carboxylate reductase

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SEQ ID NO:	APPENDIX 1 (cont.) INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTIDE / GENE
35, 36, 37, 38	C10	ribosomal protein L18a
39, 40, 41, 42, 43	C118	ribosomal protein S11
44 & 45	C122	ribosomal protein L16/L10E
46 & 47	C127	FMRFamide-related neuropeptide precursor
48	C129	ADP-ribosylation factor 1
49	C130	ribosomal protein L11
50	C137	nematode specific; conserved in <i>C.elegans</i>
51 & 52	C138	ribosomal protein L7
53	C145	ADP/ATP translocase
54 & 55	C148	troponin
56 & 57	C154	calponin
58	C16	translation elongation factor EF1A
59 & 60	C18	40S ribosomal protein S16
61	C27	ubiquitin
62 & 63	C46	nematode specific
64, 65, 66	C48	ribosomal protein S3AE
67	C59	40S ribosomal protein S5/S7



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SEQ ID NO:	APPENDIX 1 (cont.) INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTIDE / GENE
68	C8	glyceraldehyde 3-phosphate dehydrogenase
69 & 70	C82	60S ribosomal protein L30/L7E
71	C90	glyceraldehyde 3-phosphate dehydrogenase
72	C135	nematode specific
73 & 74	C206	predicted troponin
75	C227	cytochrome P450
76	C238	vacuolar ATP synthase subunit G
77	C246	40S ribosomal protein S4
78	C308	FMRFamide-like neuropeptide precursor
79	C342	ubiquitin
80 & 81	C344	nematode specific; conserved in <i>C.elegans</i>
82, 83, 84, 85	C370	40S ribosomal protein S5/S7
86	C426	nematode specific
87	C458	histone H4
88 & 89	C481	ribosomal protein L30E
90 & 91	C556	nematode specific; conserved in <i>C.elegans</i>

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SEQ ID NO:	APPENDIX 1 (cont.) INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTIDE / GENE
92	C628	ribosomal protein S17E
93 & 94	C665	malate dehydrogenase
95 & 96	C669	malate dehydrogenase
97	C694	ribosomal protein S3AE
98 & 99	C709	ADP/ATP translocase
100 & 101	C714	ADP-ribosylation factor 1
102	C721	calponin
103 & 104	C726	ribosomal protein L11
105	C736	nematode specific
106 & 107	C773	troponin
108	C834	nematode specific
109	C860	bystin
110 & 111	C863	troponin
112 & 113	C883	translation elongation factor eEF-1A
116	C888	40S ribosomal protein S16
117	C898	glyceraldehyde 3-phosphate dehydrogenase
118 & 119	C935	peptidyl-glycine alpha-amidating monooxygenase
120 & 121	C937	calponin
122 & 123	C942	peptidyl-glycine alpha-amidating monooxygenase

SEQ ID NO:	<b>APPENDIX 1 (cont.)</b> INTERNAL IDENTIFIER	FUNCTION OF POLYNUCLEOTIDE / GENE
124	C954	arginine kinase
125, 126, 127	C969	calponin
128 & 129	7235653	ribosomal protein L18A
130	8005381	neuroendocrine protein
131	7235496	pyrroline-5-carboxyla te reductase
132 & 133	7275710	protein phosphatase pp1-beta catalytic subunit
134	7923685	nematode specific
135	7641370	40S ribosomal protein S11
136 & 137	7923404	nematode specific
138	7797811	ATP-dependent RNA helicase
139	7143613	predicted phospholipase D

## Appendix 2:

### Exemplary genes used for RNAi vectors.

#### Promoters:

##### *Constitutive:*

##### **Super Ubiquitin from Pine**

CCCGGGAAACCCCT CACAAATACATA AAAA AAATTCTT TATTTAATTATCAAACCTCTCCACT ACCTT  
 TCC CACCAACCGTTA CAATCCTGAATG TTGGAAAAAACT AACTACATTGATATAAAAAAACTA CATT  
 CTT CCTAATCATAT CAAAATGTATAAATAATATCCACT CAAAGGAGTCTA GAAGATCCACTT GGACA  
 AATTGCCCATAGTTG GAAAGATGTTCA CCAAGTCAACAA GATTATCAATG GAAAAATCCATC TACCA  
 AACTTACTTTCAAGAAATCCAAGGAT TATA GAGTAAAAAATCTATGTATT ATTAGTCAAAA AGAAA  
 ACCAAAGTGAACAAA TATTGATGTACA AGTTTGAGAGGA TAAGACATTGGA ATCGTCTAACCA GGAGG  
 CGGAGGAATTCCTA GACAGTTAAAAGTGGC CGGAATCC CGGTAAAAAGA TTAATTTTTTT TGTAG  
 AGGAGTGTCTGAAT CATGTTTTTAT GATGGAAATAGA TTCAGCACCATC AAAAACATTTCAG GACAC  
 CTAAAATTTTGAAGT TTAACAAAAATA ACTTGGATCTAC AAAATCCGTAT CGGATTTTCTCT AAATA  
 TAACTAGAATTTCA TAACTTTCAAG CAACTCCTCCCC TAACCGTAAAC TTTCTACTTC ACCGT  
 TAATTACATTCTTAAGAGTAGATAAA GAAATAAAGTAA ATAAAGTATTC ACAACCAACAA TTTAT  
 TTCTTTTATTACTT AAAAAACAAA AGTTTATTTATT TACTTAAATGG CATATGACATA TCGGA  
 GAT CCCTCGAACGAG AATCTTTATCT CCCTGGTTTTGT ATTAAAAAGTAA TTTATTGTGGGG TCCAC  
 GCGGAGTTGGAATCC TACAGACGCGCT TTACATACGTCT CGAGAAGCGTGA CGGATGTGCGAC CGGAT  
 GACCTGTATAACCC ACCGACACAGCC AGCGCACAGTAT ACACGTGTCTTT TCTCTATTGGAA AATGT  
 CGTTGTTATCCCGC TGGTACCAACC ACCGATGGTGAC AGGTGTCGTG GTCGTGTGCGT AGCGG  
 GAGAAGGCTCTATC CAACGCTATTAAATACT CGCCCTC ACCGCTTACTT CTCATCTTTCT CTTGC  
 GTTGATAATCAGTG CGATATTCTAG AGAGCTTTTCAT TCAACCCGGG

##### **Strawberry Banding Vein Virus 1**

aagcttttctactgtgggttaatttcatttaattctatccaggtgaaaacctcaaggaga  
 tctctcttctcccaaaagacctctacagggcaatcaaaactacagaaccagagttt  
 gtagtgacagagttagaccaatctacctgagaatcacgagtacettcttagagtggtg  
 aaatgatgacatccttattccataccactggattgaggtaggactatccaatggaa  
 aaattccatgggacaagtcatataagaagaccgcaacagtcgagtatcttccagaga  
 taactgcactcagacctaaaaggataaaagcagtatataatcagtgactaagatct  
 tcgcagattcaagaagaagctt

##### **Strawberry Banding Vein Virus 2**

Gtttaaacacagcccaagataacagaaaagtcagaggtgttcgaaagaccacttgt  
 gactaaggatcatttcatccataat tatctggttagcacagactcatgataactgcga  
 ggaacacaagttctttacagtcgatt caaagacactttctctttauggtttcattga  
 aggagccgacccagaatatgtcagagaagcttttctactgtgggttaatttcattaat  
 ctatccaggtgaaaacctcaaggagatctctcttctcccaaaagacctctacagggc  
 aatcaaaaactacagaaccagagtt tgtagtgcacagagttagaccaatctacctgag  
 aatcacgagtacettcttagagtggtgaaaatgatgacatccttattccataccactg  
 gattgaggtaggactatccaatggaaaaattccatgggacaagtcataaagaagac  
 cgcaacagtcgagtatcttccagagataactgcactcagacctaaaaggataaaaagc  
 agtatataatcagtgactaagatct tgcgagattcaagaagaagcttaactatgc  
 tgatgacaagataattctaataagcaattattcagaattaatcaaggagaagaatt  
 aataactctttcagaatatgaagcccgctttacaagtggtcagctagctatcactga  
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 agaaaaagacaaccacagctcatcatccaacatgtagactgtcgttatgcgtcggct  
 gaagataagactgacccagggccagcactaaagaagaataatgcaagtggtcctag  
 ctccacttttagctttaataattatgtttcattattattctctgcttttgctctctat  
 ataaagagcttgtattttcatttgaaggcagaggcgaacacacacagaaacctccc  
 tgcttacaaacctgtattgttagctaaacctcttaggagatatac

**Nematode Inducible:****Trypsin Inhibitor from Arabidopsis (clone#6598343)**

cccgggagcaagcaaacaccagagaagaagaaaagcactacagagaaaaatgtg  
 agcttaagcgctctccaacaacacttctctgggagctctaaaggatgctgcaaaaagc  
 ctctgggtggtagacttccgcataatctccaagcatgggtttatctttgttagcacaca  
 aactatctgacctcgacttggatttctctctgcagtttgtccaactacattgaaac  
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 agttcatatccatcttatgatgtgcctaaaagaggggagaagatgcgaagacagaa  
 tttcatatttgaaagggttcgatatcgatatgggaaacgaatcaaggtcaaaaaa  
 ctcagttcaatagttgaaatttaaaaattttatttaattcaatccgattgggttcgtt  
 ttgttatgggttcgggtctatatcatcaaaccaatcgggttggtcctaagataatta  
 taaatattcaccaacaccagtggttaaacacatatcaacaaacctaaagttagataaa  
 caaagagacccggg

**Arabidopsis Transmembrane Protein from Arabidopsis  
 (clone#6468048)**

cccgggaattggcactcttcttctgcctgggttccaaaagaaacgaatcaatatgtgc  
 aacaagaagagctccagaagcagtcactcttctaaaatcttaatctaacaacagctca  
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 ggacacaaagtcacaaacctgaatggcttaagccgaactgagtggttttgactagaccat  
 catcagaaaaagctccaagacggtagtcggatgttagatcgtcgaagtaatttttgg  
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 aaaatgaggtacttgacgcatctctacattcactgtttaattagagaacaatacgt  
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ctttacacaggaaaattcccaact c t t g t g a g c c a c c a g g a a t c c t t g g a g t c a a a g  
gtgaatgaaacaaaggcaacagtgaagttccagctgaagaaggttctgtgcatggga  
gttgagtttgtaacctttccggg

**Diaminopimelate Decarboxylase from Arabidopsis  
(clone#4159709)**

ccccgggtggcaaaactgagatataagaggggaaggtgattttcatgcaaatTTTTTTT  
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aagtaacttcatttctgagtttttgaaaaatctaagacacaaaagactttacaatt  
taataaaaaataataaaaaatact t t a t c a c t c t c a a c g a a a t t g t t g a t t a a t a a  
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gataatagaaactagtgtggtacgtaaaaatacctctcatttggcaaaataacggtta  
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ggattataaggggaataagaaaaaagaaaagagagagtatataataacttttata  
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acagattgtgaatgagaccaaatacaatgggtcataaacgggttgggtttaaacgggca  
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agaatccaaaagatgggatcaaagaatataatctaattgggctgaccacattttccga  
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tgctttgatgatggtccggcccg

**Peroxidase from Arabidopsis (clone#4006885)**

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ttctccaataagtttttacaatttttaattgtttgcatttcccttgattatttatct  
tcatcccaatttagctaataccaactccgtttcttattcttccaagtcctttcctat  
aaatacgttcttctccctcttatctcatatcactcaccacaaagtccttctcattt  
cctcatcccg

**Mitochondrial Uncoupler from Arabidopsis**

(clone#4220510)

ccccgggatgttgtagtgaaggagaagaagagggaacaaaggatatttattttagc  
gagttttgttttgtagcgggtttgtctgtgttcaatgttgacgaaacgagtga  
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cttccatcgattcctggtctctctct ctcgctctctctgcatgtgctaaatcgccgg  
actgatcctcactgtcacctctgttc ccggg

**Stress protein from Arabidopsis (clone#6598614)**

ccccggggttaggggtttgagttgtc actggaagagggtttgattgtgagtgtgat  
ggagagattatgaaggagtttgtgtgt attttatagaggagttagggttttgagggtt  
gatgagaagtaggtttgaagaagttt tgttggtgcaactatttagagttacttgtt  
ccacaaccacaagtaagattggtcac tcttaagttctaactagaaacaacctgaca  
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tatgaattcaaaccctgaattacttt gacgaggacccgacgggtataaatagggtgat  
ctcccaacaacaaaaagggtcccggg

**Pectinacetyl esterase from Arabidopsis**

(clone#6671954)

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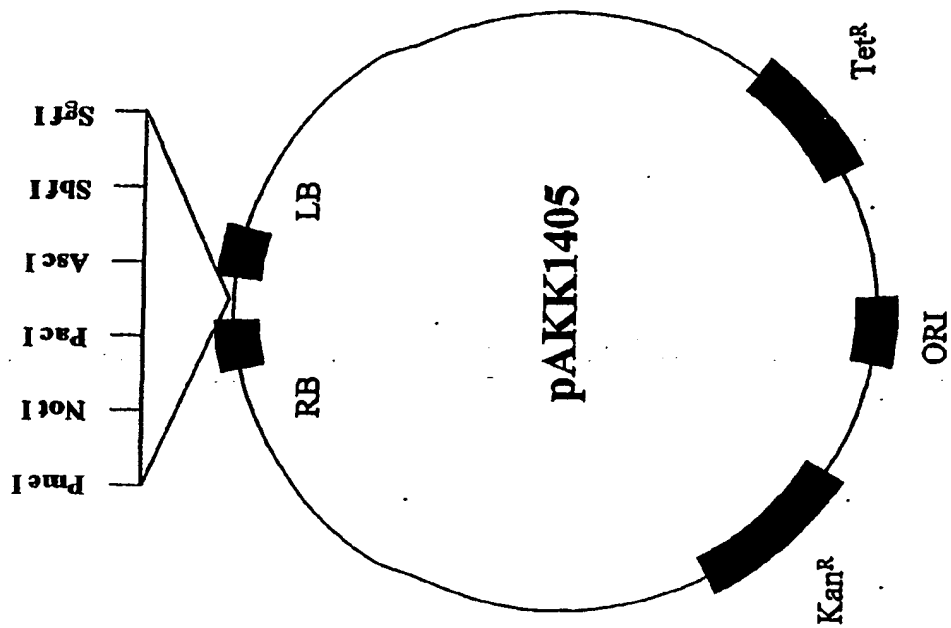


FIG. 1

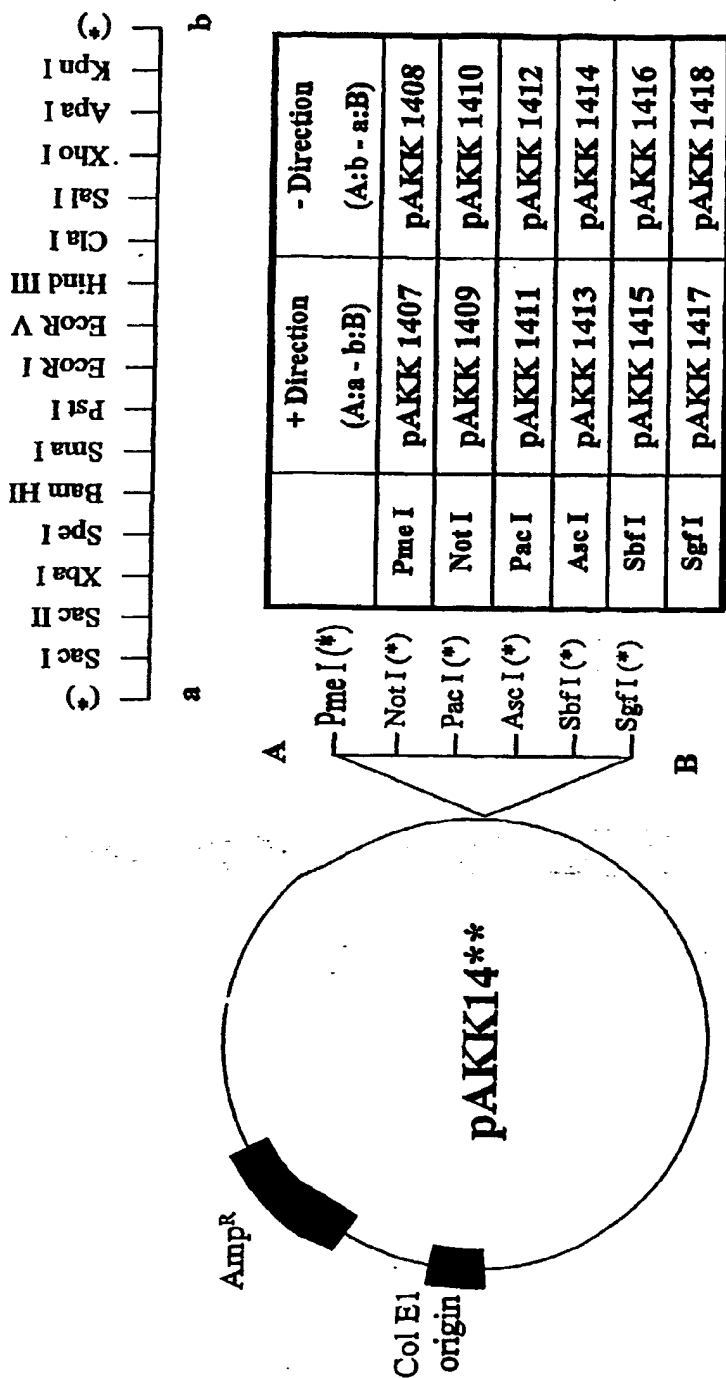


FIG. 2

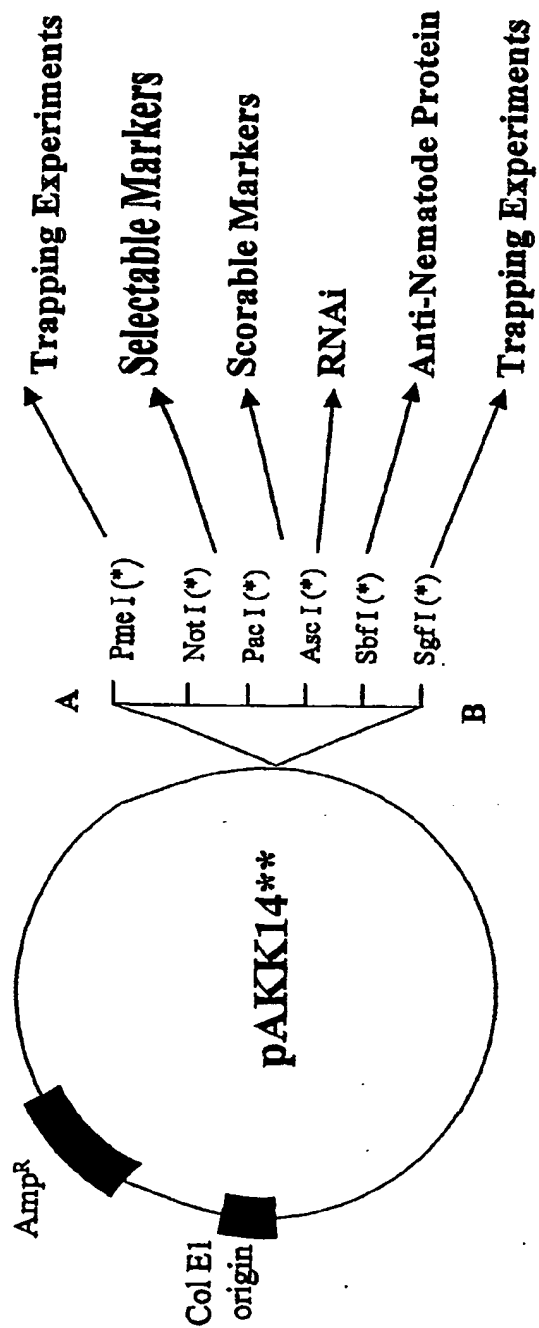


FIG. 3

# Selectable Markers

pNOS / NPT-II / tNOS

pSU / Bar / tNOS

pSU/ Intron / Bar / tNOS

pUBQ3 / Intron / PMI / tNOS



FIG. 5

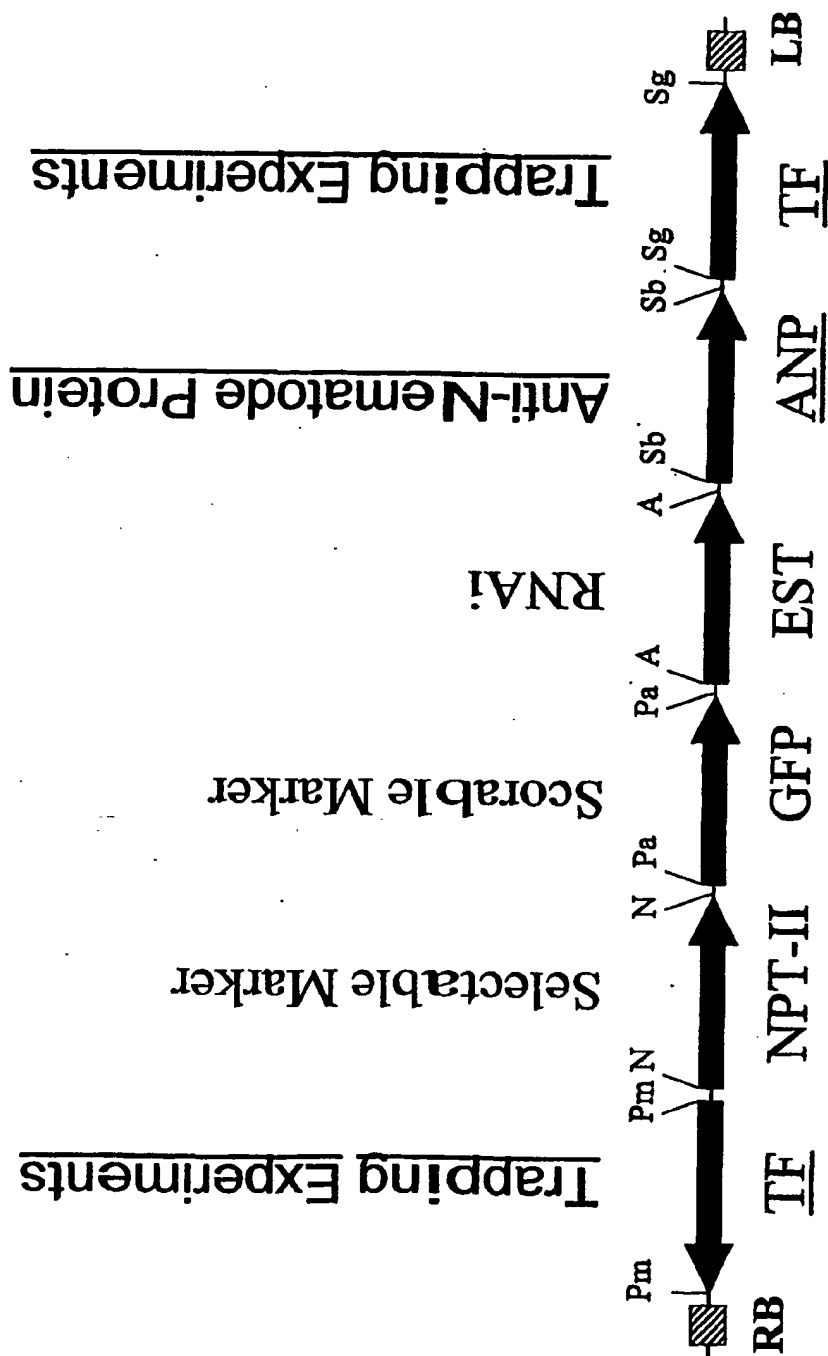
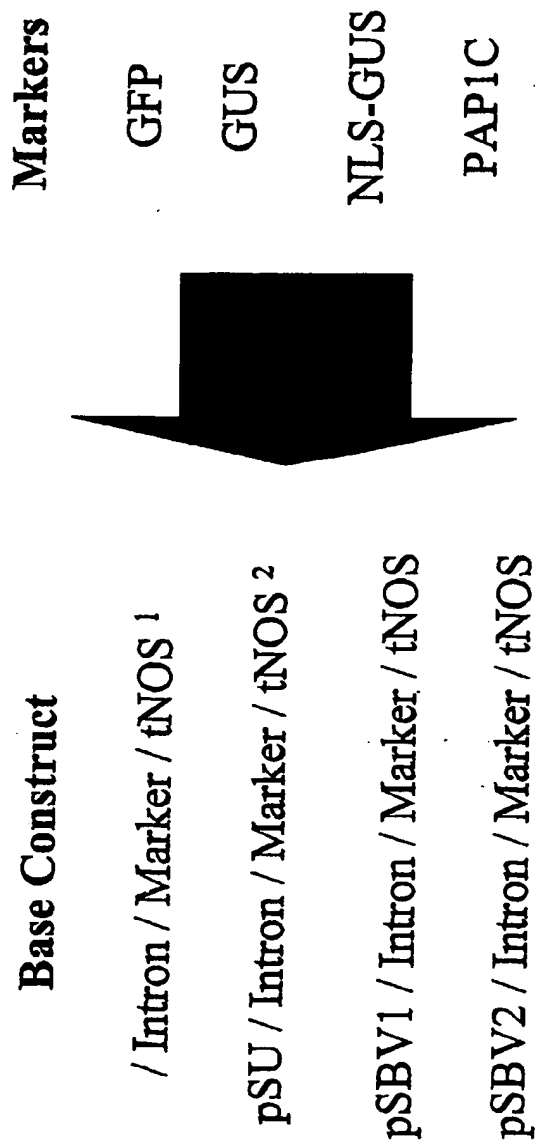


FIG. 4

## Scorable Markers



<sup>1</sup> Construct useful for promoter analysis.

<sup>2</sup> Construct useful for high constitutive expression of genes of interest.

FIG. 6

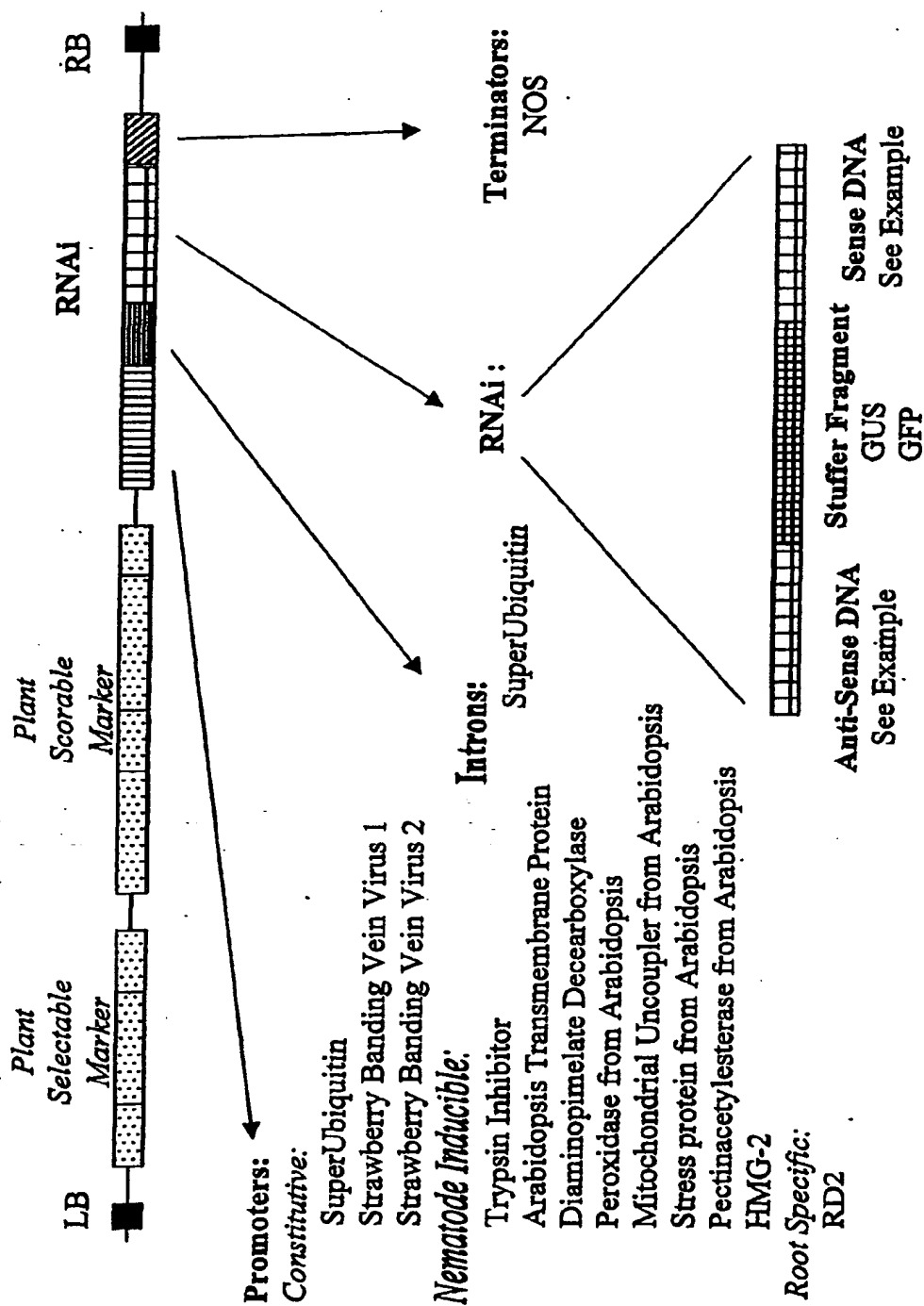


FIG. 7

AKK110P1  
SEQUENCE LISTING

<110> Mushegian, Arcady R.  
Taylor, Christopher G.  
Feitelson, Gerald S.  
Eroshkin, Alexey M.

<120> Materials and Methods for RNAi Control of Nematodes

<130> AKK-110P

<140>

<141>

<160> 139

<170> PatentIn Ver. 2.1

<210> 1

<211> 165

<212> DNA

<213> Globodera rostochiensis

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<210> 2

<211> 342

<212> DNA

<213> Globodera rostochiensis

<400> 2

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ttcgacaagc gccggcaatt tggtcgttga gaaagagggg aaggccacgc acaccatcaa 120
ggtgttcaac ctcaaggacc cggccgagat caaatgggct gaggtgggcg cggaatatgt 180
gacgagtc accgggggtgt tcactaccat tgagaaggct tcggcacact tgaagggggg 240
cgccaagaag gtggcatct ctgctccgtc cgtgatgca ccgatgtacg tgatgggcgt 300
caacgaggac aaatatgacc cggccaaggga caacgtgatt ag 342
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<210> 3

<211> 205

<212> DNA

<213> Globodera rostochiensis

<400> 3

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gaagccggcc tcattggacg ccatcaaggc ggcgggtgaag aaggctgccg aaggggaattt 60
gaagggcatt ttgggttaca cagaggacca ggtggtgtcc acggacttcc ttggagacag 120
tcgtcgtcg atcttcgacg ctggggcgct catctcgttg aaccgcact ttgtcaagtt 180
ggtcagctgg tacgacaatg aattt 205
```

<210> 4

<211> 167

<212> DNA

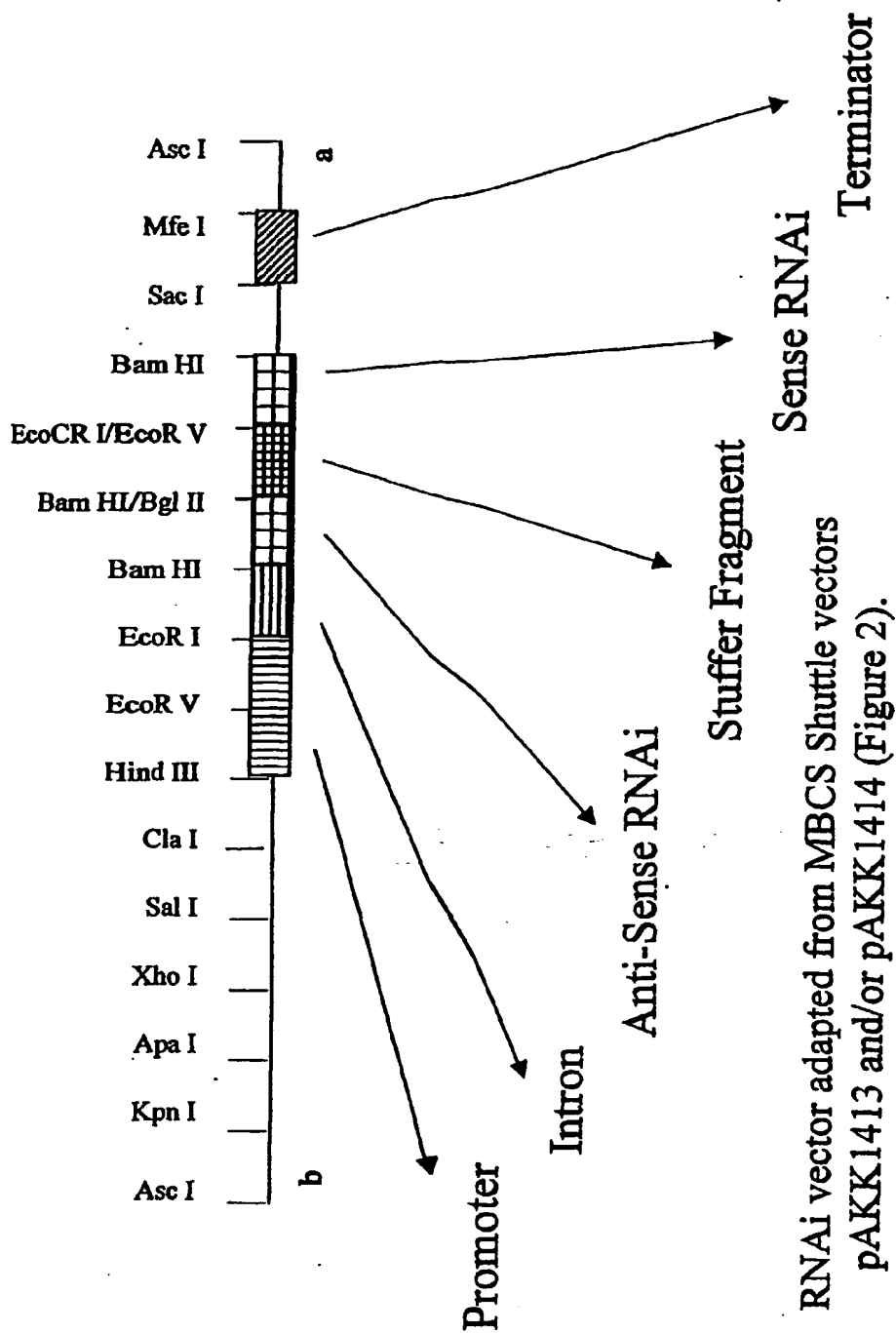
<213> Globodera rostochiensis

<400> 4

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ttaaagcatt tattcacacg cacggagaaa tgaggattac ctaatttgat tgagtctttc 60
tcgtccattt gtcaattgtg gccctaaaga gggccggttg ggtagtttt ttggtgttcc 120
ttctccttgc tggctcaacc accgaagcag tacagcgtcc ggccttg 167
```

<210> 5





\* RNAi vector adapted from MBCS Shuttle vectors  
pAKK1413 and/or pAKK1414 (Figure 2).

FIG. 8

## AKK110P1

<211> 41  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 5  
 catggccgtc acggtcttgc gcttcgcgtg ttcgcagtat g 41

<210> 6  
 <211> 79  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 6  
 gtttcccagg aaaactttca gcacggaaCg agtctcctcg taaatgaggc cagagatgcg 60  
 cttaacgcct ccacgacgg 79

<210> 7  
 <211> 168  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 7  
 cggcttggtg atgccctgga tgttatccCg caagactttt cggtgccgtt ttgcgcctcc 60  
 ctttccgagt ctttttccgc cttttccgCg tccggacatt ttgttgtaa atcagaagag 120  
 cacagagagt aggagaaata ggaaattttg cctcgtgccg aacgtgcc 168

<210> 8  
 <211> 330  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 8  
 gacagtctcc gttctggtta tgtgtcacac gcgcgaactt gctttccaaa tttctaagga 60  
 atacgagcga ttcaccaagt acatgccggg agtgaagggt tccgtattct tcggagggat 120  
 gccgataaag aaagacgaag aggtattggc taagaacacg ccgcacattg tcgtcggaac 180  
 gccgggacgt cttttggcct taggacgcac tggacatctg aagctgaaag gcgtcaaadc 240  
 ctttgtgctg gacgaatgcg acaaaatgat tggagatgcc gacatgcgcc acgacgtgca 300  
 ggaaatcttc aaaatgacgc ctgaggagaa 330

<210> 9  
 <211> 136  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 9  
 actttgccgc gggagctgcg cgtcttctgc aaaaagtcca tgcaggaacc aatggaggta 60  
 tacgtcgacg acgaggctaa gcttacgctt cacggctctc aacaatacta cgttagactg 120  
 aaggaaaatg agaaga 136

<210> 10  
 <211> 141  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 10  
 tattaaaaa aaatacaaac aataatataa tggctgtttt ttctgtcatg tttcaagttt 60  
 ttgttgttca tcactttctt cagcagcgac aatacggcca atccgggtgaa agggccaaag 120  
 tcaatagctc gctcgggtacc t 141

<210> 11  
 <211> 141  
 <212> DNA

## AKK110P1

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 17

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tcaagta cgc gcagtcgtac aacgaagcgc c gcatgatctg caaacagcgg ctgatcaagg 60
tggacggcaa agtgcgcacc gagatgcgc t tcccgtgcgg aataatggat gtgatctcga 120
ttgagaagac aaacgaacg tttcgtctgg g tgtacgatgt gaaggccgt tttgtcatcc 180
atcgaattca aaagctggag ggccagtaca a agctgtgcaa agtgaagaag caggccgtcg 240
gggacaagca ggtccccctac attgtcaca c atgacgcgcg caccattcgc taccggaccg 300
ctcatc                                     306

```

&lt;210&gt; 18

&lt;211&gt; 528

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 18

```

gaattcgcac aacgaattga agacttatg c ggcagaaaaa ggacttttgc caaagtgtga 60
ggagcaagca gacgaccttt cggattggc t ttgttcgtcc attgggttgg agcatcgccc 120
gttcctaccg tatacaaacg ctgtaataa a tgaaacaatt cgattagtca atttgatccc 180
gttcaatctt agccatttgg cgcttgaaga a tatgcaaatt ggcaatttta ttgtgaagcg 240
tgggacacca attgtaccgc aggtcagca g tgttctgttc gacgaaaaac tgtatccgga 300
gcccgatcgg tttttgccc aacgctttc t ggacgatgag ggccgtttga agaaaagcga 360
cgaacttatt gcatttgggg ttgggaaaaa g gcaatgtgcc ggcgaaagctt tggcccgaat 420
gacacttttt ctgtttgccg ctaatttct t tctcgccctac aaagtcttcc cgtccgatcc 480
actgaatcct ccaagcctga aaaagttgg c ggattatctg tttacaca 528

```

&lt;210&gt; 19

&lt;211&gt; 335

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 19

```

gaattctttg agaaagcggg aattcgttt t tggctataaa atgattctgt gggccacgat 60
ttgttgatg gctttggaca ttgcgttcg g tggcaccaat caaatggaat ttgatcagtc 120
ggcgccgatg ttccccgact cccagttca t cgatttgatt tcgcgcgaca tcgaatcctt 180
ctccggccca ttgggcgttg gccataaat t tatgagcggc ggtgcccgtg agggcgcca 240
acagctaggc cccgaggggc ctttgagca a gcggaacag gtgaagagtg acaatgttct 300
ccccgcgtat tgcgagcctc caaatccct g tccga 335

```

&lt;210&gt; 20

&lt;211&gt; 52

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 20

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ggacggctgc acggaacagt tcgagaaca c tgccgagttt tcgcgcagct ac 52

```

&lt;210&gt; 21

&lt;211&gt; 190

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 21

```

gcttgtgtga ccaggagcac atgtttaac t gtcgctcgaa gaacaaccgc gaggagtacg 60
agcaggatct ggagcaattg ctggccaaca a acggactgca caaatcaatg attgccaaga 120
aattccatct cacgcgggag gagggagccg c gccgtcgaaa acgctcttgt cgcccggctt 180
cggccaaccg                                     190

```

&lt;210&gt; 22

&lt;211&gt; 52

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

## AKK110P1

<400> 22  
ccgctacaac ccctacctgg agggcgcccc gctgaagtca gtggccaaaa ag 52

<210> 23  
<211> 54  
<212> DNA  
<213> Globodera rostochiensis

<400> 23  
gaattccgac tctcaagggtg gaccacgccc caaccaacag caattgtcag ctgc 54

<210> 24  
<211> 77  
<212> DNA  
<213> Globodera rostochiensis

<400> 24  
ccgcacatgt cgaggcctcc atcttttggc actgggtcatc accttccgcc tactgctaac 60  
aacagaccgg aacagca 77

<210> 25  
<211> 439  
<212> DNA  
<213> Globodera rostochiensis

<400> 25  
gtcaatcaaa aacgccgact tcgattcctc agctgatggt cagtaatgcg ctaaagggca 60  
tccattccgt ctctctaca tcagcaacac aatcacattc cagccccagt tttatgacac 120  
acaacgtgca gcagcaacat gttgttggtc aacaacagca gcaacaacag aatttccaac 180  
aaccgccgcc cctatcgtac actcacagcc accaacaaca aaaacaacca ccacaagcgt 240  
cacagtcgat gttgtcaatg aaaagtggca atgttgtcgt tgttgttccg caacaatcgc 300  
agcagcacca ctaccaacag cggacactga cgccactgaa gcacacatcc gcctcctcca 360  
cgtccgatcg cttcgtcatc accaaaaaca acagggtgct tccactcccg tcgcagcaag 420  
gcgcacaggc cactgatga 439

<210> 26  
<211> 539  
<212> DNA  
<213> Globodera rostochiensis

<400> 26  
gaattcgttt gagacacatc caattaatta atagttattg ttggcaatgg gacgagttcg 60  
aacgaaaact gtgaagaagg cgtcgcgcgt cattattgag aagtattaca ccaaatggg 120  
cctcgacttt cacaccaaca agcgcatttg cgaggagggtg gccattatcc caagcaaacg 180  
gatgcggaac cgaattgcgg gatttatcac acatctgatg aagcgatttg agctggggccc 240  
tgtccgtggc atttccatca aattgcaggga ggaggagcgc gagcgtcgcg acaattacat 300  
gcccgaatc tcttacctgg atgcgcagaa tcaccagatg atcagcaccc accaagagac 360  
gaaggatatg gcggaatttc tggggctagg cctcaacttg gaagtgaag ggcctttgac 420  
gagtgccggc gctggcgag gacgtcgttg agtcaggaca attggcatta ttgttgaaaa 480  
atcatcgatg tttgttcgc atttggatga taatgcgctg ataaattttt gttgatttt 539

<210> 27  
<211> 179  
<212> DNA  
<213> Globodera rostochiensis

<400> 27  
gaattcnaca gtttctgtga gtaatggcat ntcacactgc cggcatccaa cagttgcttg 60  
cggccgaaaa gcgtgcggca gaaaagatta atgatgcccg gaagcgaaaa gcacagcgac 120  
ttaagcaggc caaacaagaa gcccaggcgg agatcgagca gtatcgnacg gagagggag 179

## AKK110P1

<210> 28  
 <211> 133  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 28  
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 gtcgctggag gcaatgaatc gcaatgtcg ggcgaacaaa cagcaggtca ttgtacgtct 120  
 gctgcagttg gtg 133

<210> 29  
 <211> 482  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 29  
 gaattcgtga aatcaaaagc tttttaatt tatttacaca aaaaatggtt ccaccaccaa 60  
 ttccgctggt ggtcactggt gccgctgga aaattggcta ttacttgggt ctgcaaatcg 120  
 caaaaggcga tgtgtttggc aaagatcag caattgttct cgttctcttc gacattccac 180  
 cgatggccga agtactctct ggtgtccat ttgaattgat ggactgtgcy ttggcaaac 240  
 ttgccggtgt ggaggctgtg accacggaa agcaggcctt caaggacatt gactacgctt 300  
 ttctgtcgg agcgatgccc cgaagagag gaatggaacg aaaggacctt ttggcggcaa 360  
 atgtcaaaat tttcaagtcc caaggcgaa cattggcccg cttttccaag cccgtncgtc 420  
 aaagtctctg tgggtggcaa cccggccaa acgaacgcgt acatttgcg aaaatatgcc 480  
 gg 482

<210> 30  
 <211> 605  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 30  
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 ctgacctta cttcccccga aaaaatggag tcagcggcga tgtttcaagc aactcgtgtg 120  
 tttctgcca ccggcacacc gtcacaatgc caaagggtca acactttggt gctgttgcca 180  
 cgactccgtg atgagattga cgagtacaa aagctaaact ttcatattgt tcatgtcttg 240  
 tttaaagcaa tgttcaagcc ggccggatt ttttaaggga ttattttgcc tctttgcaaa 300  
 tctggcactt gcactctccg tgaagccat atctttgggt ctgctctgcy aaagatttca 360  
 ataccgcaac tccacgcccg tcagcaatg ctacgcatag caaaaatgga ctactcgggc 420  
 gccatttctt ttatcttacg tgttcttgt gaaaaaaatt acacacttcc tttccgagca 480  
 ttagacggcc tcgtttttca tttctttgga atgcgctcac atcagggcga gctgccagt 540  
 atttggcacc agacactgtt ggcttttgt gagcggttac caaaagacat aagtgcagaa 600  
 cagag 605

<210> 31  
 <211> 112  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 31  
 ccattcccat catcaaatta ccccgattta ctgcggcttt tgcgcggcgc cgagtcgagg 60  
 aatgaggaaa gtgaagcaaa tgtgccgtt tatgcgcgta atgatgaaat gg 112

<210> 32  
 <211> 105  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 32  
 gaattcgttt gagcatttat ttgacaaaa ctgaataaat ggccgtacca aaagaagtta 60  
 ttgacaaaat cgaggcgggt tacaagaag ttcaggaagc gtctn 105

<210> 33

AKK110P1

<211> 425  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 33  
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 gcgaccttgc tggatgtgat ccagtcggg c gttgccaaact tggacagcgg agttgggggtg 120  
 tacgctcctg acgctgaggc ttacaccttg ttcaagccgt tgttcgacc gatcatcaac 180  
 gactaccatg gtggcttggg tccgggcag c aagcagccgg caactgacct tgggtacggc 240  
 aaaacgcana tgctgaccgg atctcgacc c cgagggggaaa atttatcaat ttcgacacgc 300  
 gttcgtttcg gccgtttcct ttaagggata cccggttcaa cccgtgcttg acnaaaggan 360  
 aactacnttt ggagatggga aacnaaggc c nagggccggt ttctaacatt ttnaagggcn 420  
 atcct 425

<210> 34  
 <211> 581  
 <212> DNA  
 <213> Globodera rostochiensis

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 tttgacggtc attccaagca gccataaa c caccaaaacc aaataccccc cccaatcga 180  
 tccccccct ccaattcctc cgcattatt c gcattatcaa ttctaatacag cacaaccact 240  
 gcatcattcc tttcccgacc atacgatgc t aagtgaact ttgaaaattg gcttcattcg 300  
 agccggaaag atggcccaag cattggcaag aggacttatc aattcggggc gatacccggc 360  
 agagaatttg atggcgagtt gtccaaaga c ggacgaggct ttactggagc aatgcaaaaa 420  
 attgggaatc ggaacgacgc acgacaaca c tttggtcgcg cgagagaacg acgtcatcgt 480  
 attggcggtc aagccgatgc acatcagca a agtgacgtcg gaaatcgac ccaatttcg 540  
 gagggaaatc ttgcttattt cattgattag gaattacact t 581

<210> 35  
 <211> 102  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 35  
 gaattcggtt gagaatttta ctttatataa ttgacgttta atcagcagcc ataagcaatg 60  
 cccatcaaag catccggaga aacattaagg aagtttattg tc 102

<210> 36  
 <211> 34  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 36  
 tgcaaatgat gcaaacccca cgcttcacaa gatg 34

<210> 37  
 <211> 100  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 37  
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 aacacacgga gagatcggtt cgtgtcaaga ggttttcgag 100

<210> 38  
 <211> 176  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 38

## AKK110P1

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 gcgagtatcg ctgatgttac cgaggccggt gccgtgaccc aatgctatcg cgacatgggc 120  
 gctcgtcacc gcgctcaggc ggatcgaa<tt caaatcatca aagtgcaaac ctcaag 176

<210> 39  
 <211> 155  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 39  
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 agcgcgcgtt ccaaaaacaa ccgatcgt<tt ttctgaacga caagttcaga acgcaaggga 120  
 ttgggaagaa ggcattccaac aaggaccgt<tt actgg 155

<210> 40  
 <211> 35  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 40  
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<210> 41  
 <211> 70  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 41  
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 gcggacgatt 70

<210> 42  
 <211> 85  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 42  
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 cgtgcttccg agatgtctct ctccg 85

<210> 43  
 <211> 193  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 43  
 agttcgggtc aatgtgctca aggtgatcaa agcatcgggc tcgaagaaag cgttcgacaa 60  
 attctgagtc ggccaagcca accgcgaa<cg gtcatttgtt atggttccta attgttgctg 120  
 tttttcaatt atttgtgtta aatgactgaa tttatgatca acggtatact agtattcttc 180  
 tgaaaaagct cga 193

<210> 44  
 <211> 219  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 44  
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 gaagacgtcc ggcgcgttgt tatcgtata<ta ttaagaacaa gccgtatccg aagtcgcgct 120  
 ttgttcgcgg tgtaccgcac ccaaaaatt<c gcatttttga ttgggtaga aagcgcgcca 180  
 ccgttgacga attcccatgc tgcgtgcata tgatatga 219

## AKK110P1

<210> 45  
 <211> 489  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 45  
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 aaggacgggt ttcataatgc cgtcagaatc catccatacc atgtaattcg catcaacaaa 120  
 atgtgtcctt gcgctggtgc ggaccgtctc cagactggga tgcgtggtgc gttcggaaaag 180  
 cctcagggac tcgtggcgcg tgctcagcat gggtgatatgc tgatgtcagt gcgtattcgt 240  
 gaccaacacc aagctcacgc attggaggcg ttccgctcgg ctaaattcaa gttccctggg 300  
 cgtcaataca tcgtcttgc ccgcaagtgc ggcttcacca aattcgatcg cgaggatatac 360  
 gagaaatacc gcaaggaggg ccgtgttatc cctgacgggtg tgcattgcaa gttactcaag 420  
 caacacggac ccgctgaagg agtggctcaa gaacccatt taatcttctg tttgtcttgt 480  
 gactcttgg 489

<210> 46  
 <211> 101  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 46  
 gaattccccg gctcagagccg ggttgacgat gtcctcctcc acctcctctc actgcgttcc 60  
 gtctctcttc agccggaaat tgttctgtg gctgttgccg g 101

<210> 47  
 <211> 485  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 47  
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 tcgttccgat gacgtcgttt ggccaaccgt tgccccctc accgctttca ctggtgcaa 120  
 acccgccgtc ttattttgtg ttcccagaaa acttgccgtt ggagcggccc ttcgacgagc 180  
 aaaacgacgg ctccgaggag gaattagccg aagaagcgat gggaacgaag gcgaagaggg 240  
 cgaaaacggtt cgtccgattc ggcaaaaggg cgcaaacatt tgtgcggttc ggaaagcgtg 300  
 cacaaacatt tgtacgcctc ggaaggga caaagggca attcgatggg aaaatgcaaa 360  
 gtgaacagca acagaaaaag gcttaaagca aacggcggcg acttttctt taatgaatgc 420  
 gcgcccaccg catgacaatt cttttgtgta atgtgttgcg atttttatga tcggtaaatg 480  
 taaca 485

<210> 48  
 <211> 651  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 48  
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 ctgctgga aa gacgaccatt ctgtacaagt taaagctcgg cgaaattgtc accaccatcc 120  
 caacaattgg cttcaacgtg gaaaccgtcg aatacagaaa catctcgttc actgtttggg 180  
 acgtgggtgg tcaagacaaa attcgtccac tttggaggca ctacttcag aacacgcaag 240  
 gactgatctt cgtcgtggac agcaacgatc gcgagcgtgt gggcgaggcg cgtgaagagt 300  
 tgatgcgaat gctggcggag gacgagttgc gcgacgcggt gttgctggtg ttcgctaaca 360  
 aacaggattt gccgaatgcg atgaacgccg ccgaactgac agacagactt ggactgcaca 420  
 acttgcgaaa ccgcaattgg tacatccagg ccacctgcgc gacttcgggc gacggactct 480  
 acgagggact ggactggctg agcaaccagc tcaagaacag aggctaagct gggttggtgt 540  
 ctgttgact tgcgcggga attgatgacg attgaattta tttgtgtgt tgcgcgcgca 600  
 gctcttttgt gggacgtccg attaattttg ataattattt tattccgtgt t 651

<210> 49  
 <211> 660  
 <212> DNA  
 <213> Globodera rostochiensis



## AKK110P1

&lt;400&gt; 49

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gaattcccaa gtttgagatc aattcagttt cacttagaca aaaatgccgc cgaaattcga 60
cccaactgag atcaaaatcg tgtacctgcg ttgcgtcggg ggtgaaattg gtgcaacatc 120
tgcaattgca ccaaaaagttg gcccaattgg attgtcgccc aaaaaaattg gtgaagacat 180
tgcaagggcc acacaggact ggaaagggtt taagggtacc tgcaagctga caattcagaa 240
tcgtgtcgcc aagatcgacg ttgtcccatc ggccgcctct ctgatcatca aagagttgag 300
cgaacctccg cgagaccgca aaaaagtcaa aaacgtgaag cacaatggca acctgacct 360
cgagcaagtg atcaacattg cgcgtcagat gcgcctcgt tcaatcgac ggaagttgca 420
gggcaccgtg aaggaaattt tgggaaccgc ccagtcggtt ggctgcacca tcgatggaca 480
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aagaaaggac ggcgcctccg atttttgtgg gacggacatt gggaatttga ggtgaatgag 600
ttgccaaatt cattcattca tcaattgttg ttattgntgg tacggataaa ttgttaattg 660

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&lt;210&gt; 50

&lt;211&gt; 625

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 50

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tacatgaaca tgcgtacccg ctcttctccc gtgccaaatt tccgcatcta ctggggcgc 180
atcggaccgt acagaccttc gttgcccgtg tacacttaca acacttacca cgggtacttc 240
ccctaccgca actaccgagg ctacaccttg gcgaatgctt actggtacga ccgatactat 300
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ggatatgctc ggccgtatca ctaccggtcc catgcgctgg cccaccggtt caattaccgc 540
gaaggaatgg tcaggaaacg ggtctgacaa atcgaaactgc tccaaattga cgtggtccgc 600
attcgaaaga agacgaaaaa agctt 625

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&lt;210&gt; 51

&lt;211&gt; 402

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 51

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gaattccaag tttgagcaac attttgaaaa tgaccgaagc caaaaaactt cccgaggtgc 60
cggaaacttt gctcaagcga cgcaaaatca gagctgcgca aaaggccgca aaagcaaaga 120
acaaattgag ttctatcaaa aaagcacgga ccaagaagggt ggaaatcttc aaaagagccg 180
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cgaagaaagt cggcaattat tatgtgccag aagagcccaa actcgctttt gtggtccgaa 300
tcaaaggcat caataagatt catccgcgtc ctgcgaaggt tctgcagctt ctccgcttgc 360
gtcagatcaa caacggcgtt ttcgtaaaag tgaacaaggc ga 402

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&lt;210&gt; 52

&lt;211&gt; 433

&lt;212&gt; DNA

<213> *Globodera rostochiensis*

&lt;400&gt; 52

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aacgcggtta cgccaaagag aagggaagc gcattccaat aacggataac aacattgttg 120
agcgcagttt gggcaagcat gacgtgattt gtgtggagga tatgatccat cagatttgga 180
ccggtcggac cgcaattcaa acaggtgaac aacttcctat ggcctttcaa gctgagcaac 240
ccggtgggcg ggttcaagaa gaagtccaat cacttttgtg gagggaggcg attatggaaa 300
ccgcgaggac caaatcaaca aattattgga aagaatggct taatgggaag gaagcggana 360
aagaaaggaa attgnggcgt ttttctgttg ttgttttgac gataaattgt taactccaaa 420
aaaaaaaaaa aaa 433

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&lt;210&gt; 53

&lt;211&gt; 768

&lt;212&gt; DNA

AKK110P1

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 53

gaattcgttt	gagggtcaaac	tttattagcg	tatttaacaa	tgtccgaagg	aggagcgaaa	60
aagagtagca	gcggtgccaa	gggggggttt	gatgtcaaga	aatttgcgat	cgatcttgcg	120
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attattgacg	tgcttgtccg	tgtgccgaaa	gagcagggct	ttctgtcact	gtggcgtggg	300
aacttggcca	acgttatccg	ttatttccc	actcaagcgc	tgaacttcgc	cttcaaagac	360
acatacaaac	gcattctttac	ggagggactg	gacaaaaaca	agcagttctg	gtcgtttctc	420
gtcatgaatt	tggcctctgg	aggtgcggc	ggcgccacgt	cgctgacctt	tgtttatccg	480
ctgggacttt	gcccgtacgc	gtttggccc	tcgatgtccg	aaaagctggg	tcccgcgagt	540
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gctttgacac	cgcgaaagatg	attttcgcg	cggatggcaa	gcagatgaat	ttcttcccca	720
catgggcat	cgctcaggtc	gtcaccgtg	cgtccggtgt	cctctcct		768

&lt;210&gt; 54

&lt;211&gt; 338

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 54

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cggtcccaa	attgaggagt	atcaacgctt	tttcgacatg	ttcgaccgcg	gaaagaatgg	120
ttacattatg	gccacccaaa	ttggacaaat	tatgaacgcg	atggagcagg	actttgacga	180
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cgacgagttc	tgcgcggttg	tgtacacggt	ggccaacact	gtggacaagg	acactctcgc	300
aaaggagctg	aaggaggcat	tccgactctt	tgacaagg			338

&lt;210&gt; 55

&lt;211&gt; 267

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 55

gaaattcgcg	ccgatctcag	cgacaaggat	ttggaggcgg	cggtcgcagc	aattgacgag	60
gacggcagcg	ggaagatcga	attcgaggag	ttctgggagt	tgatggcggg	cgaaaccgac	120
tgagaaaaga	gcaaatcgat	ccaaatccaa	acggagcccg	cccatttcac	ctccatccgt	180
ccgtcgtaatt	attatatatt	ccagtggaa	tttcccat	aaattcggtg	aaagtaaaat	240
aatttgacga	aaaaaaaaa	aaaaaaa				267

&lt;210&gt; 56

&lt;211&gt; 597

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 56

gaattcgctg	gacacttcgc	atccggagta	cagccacgag	cagagcatcg	accagaccag	60
catccccctac	cagatgggtt	cgaaacagta	cgCctcgag	aagggcatga	ccggctttgg	120
acagccccgt	tgggaggtgc	ttgaccgct	catctcgtac	cagaaccgca	agtcgcaagg	180
aatgggtcgt	ctacagtcgg	gtaccaaccg	gttcgcctcc	caggcgggca	tgaccggctt	240
cggcacaccc	aggaacacca	cctatgaggc	ggaggcaggc	gagctgccct	acgaggacat	300
gaagaagtgc	gaggcgatca	tcccgtccca	ggccgggttg	aacaaggcg	actcgagaa	360
gttgatgacc	aacttcggca	cgcccgtaa	caccaccacc	aaggtcaaag	tggagaattt	420
ggcggaatt	ccggaggaca	ttttgtgaa	aggacacggc	gaggtgcgcc	tgacgtccgg	480
taccaaccgg	ttcggtccc	agaagggtt	cgtcgcgttc	ggtaccggac	gtgacgtgtg	540
ccgtgagggg	gtgaacgtga	acgtgtgc	gggcgacttg	gagccgcttc	cggagga	597

&lt;210&gt; 57

&lt;211&gt; 80

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

## AKK110P1

<400> 57  
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<210> 58  
 <211> 513  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 58  
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 ttgtcgaact gattccgacc aagccgatgt gtgtggaggc attcactgac tacgcaccgc 180  
 tcggccggtt tgcgtttcgc gacatgaggC anactgttg cgtgggcgcg atcaaatcag 240  
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 gtggcgggaa gaagacatga ccaaggggaG gggcggttcc ctaagggcca accgtcgacg 360  
 aaaatgcgac caacctctt tttatcgttG tcttattcag ttccttcac cgtctctat 420  
 ccatattgtc gttgcgttgg ataattgttT atttttgtt attgtcctgg ttggaaaata 480  
 aatttggtca attaaaaaa aactcgtgcC gaa 513

<210> 59  
 <211> 393  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 59  
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 aaaaggggca agggcttgat caaggtcaat gggcgctcct tggactacat gcagccggag 180  
 attctgcgca ttaagctcca ggagccaatt ctcatgttg ggaaggacaa atttgagga 240  
 atcgacatag gaatccgcgt caagggcggT ggacacattg cgcaaattha tgcaattcgc 300  
 caagcactgg ccaaggcact ggtcgcttC taccagaaga atgtcgacga gcagagcaaa 360  
 aaggaactga aggagcaatt tgttgcttaC gac 393

<210> 60  
 <211> 154  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 60  
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 taagaaataa tttttagat caaatgtttT gatgatgac cttgttttg ttgttgataa 120  
 aaaaaattta taataaaaaa ccgccgataC tgac 154

<210> 61  
 <211> 666  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 61  
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 aactgtcatc atgcaaattt tcgtcaagaC gctcaccggc aagaccatca ctctcgaggt 120  
 cgaggctagc gataccatcg agaactgaa agccaagatc caggacaagg agggcattcc 180  
 gcctgatcag cagcgtctga tcttcgccgg aaaacagctt gaagacggac gcaccttggc 240  
 cgactacaac atccagaagg agtccactC ccactctgtg ctgctctcc gtggcggaaT 300  
 gcaaattttc gtcaagacgc tcaccggcaa gaccatcact ttggaggtcg aggcagcga 360  
 caccatcgag aacgtgaagg ccaagatcca ggacaaggag ggcattccgc ctgatcagca 420  
 gcgtctgatc ttcgccggaa aacagctcga agacgggcgc actctggccg actacaacat 480  
 ccagaaggag tccactctcc atctcgtctT gcgtcttcgt ggaggagaga actgaatcgc 540  
 gggctgatgg aaagatgacg aatatgatgt ctattcgatg acttgtctct ttcgatataa 600  
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 gataaa 666

## AKK110P1

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 <211> 213  
 <212> DNA  
 <213> Globodera rostochiensis

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 gtttgaggag acacattcgt tcgcgcaagt ggctcgaaga taccgggcag aatttggtat 180  
 ggaaccaccg cagttggacc aagtgaagaa gtt 213

<210> 63  
 <211> 488  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 63  
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 ggtcttggag aacaacagcc aattcccgct gtaagcgatg cgggactgga tgcggaagaa 120  
 cagctgagaa tggccagaat gtgagccgga ggacctgaag atttatgaac gaaattttcc 180  
 agtgaagtgg accaacgctc ttgacttta tctgcttgt gtaaagtgt tagaatcggc 240  
 ttccaattca aaggcttttc attccccaac ttttatttt gcgcaaaaaa tttcttagga 300  
 taagcgtgaa taatttattg atttgtttt tctttcttt atctccgcct cgaagtcgca 360  
 agtgttcctt ttggcccggt cctttttgt ttgaatgtta ttccattccc atccccctac 420  
 tttctcatat ttgtgacatt cagctgcatt gttcgactcc catttaaaag ttgagtgaag 480  
 tgcgattg 488

<210> 64  
 <211> 249  
 <212> DNA  
 <213> Globodera rostochiensis

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 gkgdyrbwnt msnwrmanrg artsstsgaa ttcccaagtt tgagagtaaa tattattagc 120  
 taaaaatggc agtcggaaag aataagagaa tgggcaaaaa gggagccaag aagaaggctg 180  
 tcgatccgtt cacacgcaa gaatggtacg acatcaaagc gccggcgatg ttacacatc 240  
 gaaatssts 249

<210> 65  
 <211> 362  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 65  
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 ntmsnwrman rgartsstsg tcaaccgtac tcagggaaac cgcatttcga gcgactttct 120  
 aaaaggccgc gtttacgaag tgtcactggg tgaccttaac agcactgacg ccgactttcg 180  
 aaagttccgc ctgatctgtg aagaggtaca gggcaagatt tgctgacca actttcacgg 240  
 aatgtcgttc actcgggaca aactgtgctc tattgtcaag aagtggcaca cgctcattga 300  
 ggcgaatgtg gcagtgaaga ctaccgacgg ttctatgctc cgactcttt gtatcgggtss 360  
 ts 362

<210> 66  
 <211> 128  
 <212> DNA  
 <213> Globodera rostochiensis

<400> 66  
 aatcaaatga agaagacgag ctatgcaaaa gcctctcagg tgcggatgat tcgtgccaaa 60  
 atggtggaga tcatgcagaa agaggtctct tccggcgatc ttgaangaaa gtagtcaaca 120  
 agcctgat 128

## AKK110P1

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 <211> 502  
 <212> DNA  
 <213> Globodera rostochiensis

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 ttgggtcagcc ttgacgttac cgaggtcaaaa ctgttcggaa aatgggccct taacgatgtg 180  
 gaagtgtccg acatttcgct tgtggattat attgcggtga aggaaaaggc ggccaaatat 240  
 ctgcccacac ggcgccggcg ttaccaacag aagcgcttcc gcaaggccac ctgtccgggtg 300  
 gtggaacggg tgtctttgtc aatgatgatg cacgggcgga acaacggaaa gaaactaatg 360  
 gcgggtgcgca ttgtgaaaca ccccttcgag atcatcacct gctaccggag agaaccagc 420  
 ccaagtgttg gtcaatgctg tgataaacag tgggcccnc gaagattinca cacgtatcgg 480  
 acgtgcgggc actgttcgtc ga 502

<210> 68  
 <211> 519  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 68  
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 ttaatcatta aaactacatt taaaatatac tttttagaga atgtcgtcta aaatattctt 120  
 ttctcccctt tatgcatcta tctaaccaga cttggaagca atatggctaa tcaagtcaac 180  
 aatacggcag gaatacccaa actcgttatc ataccagcta accaatttaa caaaatgcgg 240  
 gttgagaacc ataagagcct cggcgtcgaa aatagacgaa tgagtgtcgc caagaaagtc 300  
 ggtagaaaca acctggctct cagtatatcc aagaatccct ttaagcttcc cttccgaagc 360  
 agtcttaatt gcattcttaa tagcctcctt cgttgctggc ttctccaaac gagcagtcaa 420  
 atcaacaacg aaaacgtttg ggcgtcggca caggaaaagc catttcgggt aagcttccca 480  
 tccaattcat ggattgacct ttccaacagc ctttgcagc 519

<210> 69  
 <211> 218  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 69  
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 ttggagaata ttaagaaggc taaggttaaa acgcaagta tctttaaacg tgctgagcaa 180  
 tacttgattg catatcgacg taagcaaaag caagagtt 218

<210> 70  
 <211> 293  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 70  
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 taaggggaatc aacaagggtta atttaaattt gctataaagt ttaggatggg tttagacaat 120  
 tcttctcttt taatgctttc taactttttc aaaaaagtta tgattttatc acccataat 180  
 ctacaaattc ttttaattat cagatccatc ctgctcctcg aaaagtctt caacttttc 240  
 gcttgcgta aatcaacaat ggagttttca tttaattgaa taaagctaca atc 293

<210> 71  
 <211> 422  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 71  
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 caggttggtt ctaccgactt tcttggcgac actcattcgt ctattttcga cgccgaggcg 120  
 taagttttga ttttctaaga ttatatataa cctttttaat ttttcagtct tatgggtctc 180

## AKK110P1

aaccgcgcat ttgttaaatt ggtagctgg tatgataacg agtttgggta ttcctgccgt 240  
 attgttgact tgattagcca tattgcttc c aagctctggt agatagatgc ataaagggga 300  
 gaaaagaata ttttagacga cattctctaa aaagtatatt ttaaattgtag ttttaatgat 360  
 taatgaattt ttattcataa attgttttg c caaatataaa ttttttatt gataaaaggt 420  
 tg 422

<210> 72  
 <211> 374  
 <212> DNA  
 <213> Meloidogyne incognita

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 tgagccagtt aaactggact tcaacgttc c gcttattagt gattgggctg ctgcttctga 120  
 gtggcctcaa gaagaggaag ctgaggttg c acctactgca ccaattgggc agccacagcc 180  
 tcaacagcag caaactcaac aaggaggtg a ttggaactct ggtactagtg gatgggtgaag 240  
 ggcaggaaaa ttgatagaaa gagaaatta t tatggaataa atgtaataca tgttgttgc 300  
 tgatttattt gttacatata caacaagtc t tattttgttg tttatttaat aaaagttgtt 360  
 aattaaaaaa aaaa 374

<210> 73  
 <211> 120  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 73  
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 agctttcaaa ttttgtttt tgattactt t ttaaacaaga ttcaactgat ggaactactg 120

<210> 74  
 <211> 369  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 74  
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 cagcactagt ctctgatgta gttttcttca a atctcatttt taagtgatgt agaggaaagt 120  
 tagaattctg attgctatcg tcttctttc t ctctcttttaa tggctttttc aatttatctt 180  
 ctctcttttc ttgtccattc ttttcttca t tcttttcaaa aggtctcagga aattttaatt 240  
 cagaccgct ccttttaact gctgtatct a aagaaaacc tctaggcaac gtcccagttc 300  
 cactcaaagt caattttgtt aaatttttg c cagatctaag tccttcttc ttttgaacga 360  
 attgaactg 369

<210> 75  
 <211> 529  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 75  
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 aatctaaata aggccttatt ctaagtta t attttcttt tacataaacc gtcaaccctc 120  
 caagtttttc aatgcttggg ggttttaatt g gatcctctgg taataatttg taggctagaa 180  
 aaaagtgtgc agcaaaaagg aaaagcatc a ttcttgctaa ggcttctcca gcacattgcc 240  
 ttttccccc accaaaagct attagctcg t cagcttttt taatttccct tcattgtcta 300  
 tataacgttc agggtcacaaa ttttgggga t ttgggtatat ctttggatca aaaagaacat 360  
 ccgatacttg gggatcata aatgtacct t taggcaacac aaactttcca acattcaaat 420  
 ctccaaggc taaatgcccc aaattgaaa g ggactaaatt aacgagtctt aatgtttcat 480  
 taacaacagc atttgataa attaattta g gtctgtgttc caaactaat 529

<210> 76  
 <211> 449  
 <212> DNA  
 <213> Meloidogyne incognita

## AKK110P1

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 gaaaggcaca acgacttaaa caagcaaaa aggaagcgca agctgaaatt gacaaatata 180  
 gagagggaacg tgaaaaacgt tttaaagagt ttgaacataa ttacctcggc gctagagatg 240  
 atattgctgc acaataaaag cgtgaaactg atgagacgct taatgaaatg actcgtagt 300  
 ttgctgctaa taaacagcag gtaattgtt gtctacttca acttgtctgt gacattcgtc 360  
 cagaactgca tcacaattta caacttcaa ttaagcttaa tgaaaagcct gcctaatttg 420  
 tagttgattg attataaaaa tgaaattga 449

<210> 77  
 <211> 643  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 77  
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 acgtcccatt tgcgggcttc acaagcttc gtaatcgctt cctcttattt tgtttcttcg 180  
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 tccggatcca cacatcaagg ttgacgaca tgttgctgtt gatataaaca ctggaaagg 540  
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 <211> 584  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 78  
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 aaataataaa ttggaatata ataaaaatga aattgagagg caaaaagagc aattaattcg 180  
 agatttgatt gcctccttaa cacgtgaaa gcaatattca cgagattggc aacaatcaca 240  
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 acaaacattt gttcggtttg gaaaaaggg acagactttt gttcgatttg ggagagattc 480  
 aaaacatcaa cataacttgt cagatcaga gcagttaaaa actgacaaac aataaaaatg 540  
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 <211> 556  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 79  
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 gtcgcttcac caatgttgct acttctgggg cgggacgccg tcgtggccct aactccaacg 420  
 ctgcataaga gaatggctgt atcttgatga atgtatggtg atataatcaa ttaatacat 480  
 tcgactntat gaagttttct gttattcaag ataaatcttt ttgttgaaaa aaaaaccaag 540  
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<210> 80

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accatctcc

429

<210> 86  
 <211> 435  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 86  
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 actggagtag gtgtacttag tgttttagaa aaggcaaaat tagtttggtg gtttgaagag 240  
 acaattcttt ttgcacaagt agcgagaaga tatcgagcag aatttggaaat ggaaccccca 300  
 catatggatt tagttaaaaa attacatcaa cgttttctca atactgggtc tgtttctaata 360  
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<210> 87  
 <211> 501  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 87  
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 aattgttctt tagccacca atccgtaaag agtacgtcct tggcggttca acgcatagac 240  
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 aatacgtttc actccaccac gacgtgcca tccgctggatt gccggtttgg tgataccittg 420  
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<210> 88  
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 <212> DNA  
 <213> Meloidogyne incognita

<400> 88  
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 agaactagtc tcgagttttt tttttttttt tttttaanaa ttaacaattt atctcatttt 180  
 cctcttccat gaaaattaac aaaaagacga caacttaat ccataattaa catcattttt 240  
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<210> 89  
 <211> 286  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 89  
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 agtttagcct ttccagaacg aagagtcttc aacgtctgct tgtagcccaa acaatacttg 180  
 cccgatttgg taaccatggc gagacgagca ttgatatttt ctgtggactt tttctgtttt 240  
 ccaacaacca ttgtaacgca aaattaaaaa ctctttttta acaaat 286

<210> 90  
 <211> 391  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 90



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&lt;211&gt; 424

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 80

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ctac

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&lt;210&gt; 81

&lt;211&gt; 89

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 81

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attatccaca cacctattgg agctaccctt accaaggaaa atggtacgac tatgacaatc 60
caacanatta cgcgccattc ttgaccca

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&lt;210&gt; 82

&lt;211&gt; 168

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 82

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tttttttttt taaaatttat tcattaacaa atgaccttaa cagataaaac ttaacagtca 60
aaagacaaca taatttccaa ctttttcaa attatccttt ttaacgggtt gattttgcaa 120
ctcgtcccaa ttcgtccttc ttcttgatag catatgaatt gctcgaac 168

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&lt;210&gt; 83

&lt;211&gt; 67

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 83

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aattcatcag ccagacattc agcaattgtt ttgatattac ggaaagaagc ttcacgagac 60
ccagtac

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&lt;210&gt; 84

&lt;211&gt; 42

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 84

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taacacgacg aagaggcgaa acatcaacag cctgacgacg aa

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42

&lt;210&gt; 85

&lt;211&gt; 429

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 85

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tatacgagta gaatcctccc gtggtcctcc attaataaca gcgccaaaca gtatttgaac 60
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gatatcgctt aaagaccatt taccaaaca ttttaattca ggaaaatcaa ttgtagtcat 360
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gtttccaatt agattccggc actctgacta c 391

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&lt;210&gt; 91

&lt;211&gt; 131

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 91

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caacaaatta ccgcccgttc ttcgaccac gcatcagcgc atcattttca agaccttatg 120
attacacatc a 131

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&lt;210&gt; 92

&lt;211&gt; 571

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 92

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cctcaaaaaa ttcattttatt gacgaccagc agcaggttgt tgctgctgtt gttgaccacc 180
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tgctttcttg acagttttga gagaaccgat t 571

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&lt;210&gt; 93

&lt;211&gt; 671

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 93

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aatttgggga aatcatlcaa gtacccaatt tcctgatgtt aaacatgcta aagtaattaa 660
aggtggcagc g 671

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&lt;210&gt; 94

&lt;211&gt; 289

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 94

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ggctgtaaat gatgtgccgt ggatacagaa tgaatttatt tcgaccgtcc aaaagcgcg 60
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tgatcacatt catgattggc actttggaac aaaagatggc gattgggttt ctatggccgt 180
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289

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 <213> Meloidogyne incognita

<400> 95  
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 aatggccgaa gtgcttaaag gagtggaaat tgaactttac gattgtgcct tggcaaatct 240  
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 <211> 323  
 <212> DNA  
 <213> Meloidogyne incognita

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 <213> Meloidogyne incognita

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 ctgagataaa agtcattcca tggaaattgg tcaacaaac tttgccttga acctcttcac 660  
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<210> 98  
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 <213> Meloidogyne incognita

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758

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 <212> DNA  
 <213> Meloidogyne incognita

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<400> 100  
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<210> 102  
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<210> 103  
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 <213> Meloidogyne incognita

<400> 103  
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<210> 104  
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 <213> Meloidogyne incognita

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&lt;400&gt; 104

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 agtgggaaaaa ttgaa 255

&lt;210&gt; 105

&lt;211&gt; 571

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 105

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 ctccctcttc tttacatcct ataatcatc g 571

&lt;210&gt; 106

&lt;211&gt; 235

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 106

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 atttttaaga aaattagatg gaaatgctga agaaagaaaa aaattattta ttttt 235

&lt;210&gt; 107

&lt;211&gt; 702

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 107

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 tattatggct actcaaatg gggtaattat gaatgctatg gaacaagatt ttgatgaaaa 240  
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&lt;210&gt; 108

&lt;211&gt; 423

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 108

aaaattaaaa taaaagacaa acaataaaat ataaattaaa taaataatat ttaaataaac 60  
 acacaaataa actctccaaa cataattttt ttaaattttt ataacatttt gtccatttg 120  
 agaaagaaaa tgccaaagga gatgaagaac ttgttgaaga aaaaagttca aaaatatcaa 180  
 ctccctcaat tgctgctaca ttttctttca ttattccatt tgttgtaagc tcagtaactg 240

## AKK110P1

```

cccccaattgt tgtttagtc catggagaga aagcactttc cccattcgaa aatgttgaac 300
caaatgtgtc aaattgttgc tgtttagtc ctcgaagttc gttagaaaca gaacgaaata 360
aattatgagg ttgtttagtc tctgacgtt tttgattgtc tggagctggg tgaggatcac 420
caa

```

<210> 109  
 <211> 994  
 <212> DNA  
 <213> Meloidogyne incognita

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<400> 109
ttttattttt tatttgaata taatcatcac attataatta atgggaaaaa gacaaaaaat 60
tagaacaggt gctggcgatc ttgtcacaac ccctggacct cttcataaac aaattgaaag 120
gtcaaaaacta gccaaagccga aattcaagcc tttaaaacgt tcaagagaag agcaaaaaaga 180
tgaaattgaa cttgtcgatc catcgtaaaa gggcaaaatt attattaaag caaacaataa 240
attggaaaaa gatgttgtgt tcaatgagga tggagaatct gataattctg aagaaattga 300
agaagaagaa gaagacggca atgaaaagtt ggatgttgat caattagtat caaaacattt 360
ggaagattta gatgaactaa aattggatga tggcgttgaa aatgtgcaa agataataac 420
gaaattcaga taaaaataac aaagaaagtg ttataataaa agctgagttt gccgatatcg 480
acccaaaaat tgttgatctt ttacagaaa ttggtcaagt tttaaagaaa tatagaagtg 540
gacgtatttc caaagctttt aaagtatttc caacttggg tgattgggag aaaattatcg 600
aattaaactcg cccagatgat tggcggcgag ctgcaatgtt acatgctacc aaaatatttg 660
cttcaactgc taccctact caatgccaaa ggttttataa ttgattttg ttgccacgta 720
ttcgagatga tattgacgga ttaaaaaatt acatttccat atgtatcaat gcttatttaa 780
agcattgttc aaaccagctg catttttcaa aggaatcctt ttgccgctt gcaaatcgaa 840
caatttttct cttcgagaag cttgtgttct tgcttctatg cttcgtaaag cctccatccc 900
tcaattacac gcgggcgcag cattgttagg tatttctgt ttagaatata cttcttcaag 960
ggcttatatc cttcaagcat tgatagaaaa gaat

```

<210> 110  
 <211> 476  
 <212> DNA  
 <213> Meloidogyne incognita

```

<400> 110
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tttatttcga aaaaatggct gagaatatag aagaaatcct tgccgaaatt gacggctctc 120
aaattgagga gtatcaacgt ttcttcgata tgtttgaccg tggaaagaat ggctatatta 180
tgccactca aattggggtta attatgaatg ctatggaaca agattttgat gaaaaaactc 240
ttcgaataat aatccgaaaa ttcgacgcag acggcagcgg caaatcgaa ttcgacgaat 300
tctgcgctt ggtatacact gtggcgaaata ctgtagataa ggacactttg cggaaagaat 360
tgagagaagc ttttcgtctc ttcgacaagg agggtaatgg ttacatctct cgtccaacac 420
tcaaaggatt actccacgaa atcgccccag acctcagcga taaagacttg gatgcc 476

```

<210> 111  
 <211> 189  
 <212> DNA  
 <213> Meloidogyne incognita

```

<400> 111
cgaagacgga agcggaaaaa ttgaatttga agaatttttg gaattaatgg ctggagagac 60
tgattgaaat tttaattaga gatgaataaa aaattaacta aaatatattg ccataaaaatt 120
ttggaaagtg ccaaaaattg cttttttgag aatttttatt tttaacgtct aaataatgaa 180
taaattgat

```

<210> 112  
 <211> 164  
 <212> DNA  
 <213> Meloidogyne incognita

```

<400> 112
ttgaggaaat ttaatttttt aaacaaatat aataattacc aaacaacaaa aaagaatccc 60
aaaaacaaca tttttaaatc aaatgacaga catatatattg caataacgat gtgtggattt 120
tctttttttt taaataatta acatcttaag cctgtatttt cttc

```

## AKK110P1

<210> 113  
 <211> 539  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 113  
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 taacaccaac agccacagtt tgacgcatgt cacgaacggc gaagcgtcca agaggagcgt 120  
 agtcagtaaa agcctcaaca cacattggct tgggttgaat taagtcgaca ataccagcat 180  
 ctccagtcctt caaagccttt ggattgtctt caaccttctt tccagttcga cggtcgacct 240  
 tctctttaag ctacgcgaac ttgcaagcaa tgtgagcagt gtgacagtca agaacaggcg 300  
 tgtagccagc agcaatctgc ccaggatggg tcatgatgat aacctgagca gtgaattgct 360  
 tgggtctcctt tgcctgggtca ttcataaggt cagaagtgc tgaaccacgt cggatgtcct 420  
 tgacagagat gttcttaacg ttaaattcaa cattgtcttc aggaacagct tcagggagag 480  
 actcgtgggtg catctcaaca gatttaactt cagtagaaat tccttcagga gcaaaaggta 539

<210> 114  
 <211> 314  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 114  
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 tccgtcgatt tactgagatt ggttcttcta aatttgcca tcccgctttt gttccaagcc 120  
 cggagaatct tgaaagagta aggaatgtc cagttttggg tgttggtgct ggtgngcttg 180  
 gatgtgaaat tttgaaaaat ttggccttat caggatttca aaatattgaa gttattgata 240  
 tggacacaat tgaccttca aatctcaaca gacagttttt gtttcgtgaa cacgatgttg 300  
 gcttatacaa agca 314

<210> 115  
 <211> 200  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 115  
 ttcgaaga cg tggttaaagga tgcgtcttta ctgcacataa ttgtaaaata caagataaag 60  
 gacttgactt ttatgggcaa ttttcaatta taatttggtg actagattct attgatgctc 120  
 gaagatgggt aaacgccaca gtgtgttctt tggtcgaatt tgacgaagaa aacaagccac 180  
 ggccaggcac aattattcca 200

<210> 116  
 <211> 471  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 116  
 tttggctgaa aaaagactgc tactgctgtg gcatattcca aaaagggaaa aggattaatc 60  
 aagggcaatg gccgtccttt agaatttttg caacctgaaa ttcttcgtat taagctacaa 120  
 gagccattgt tgattgtagg aaaggacaaa tttgctggaa tggatattcg catccgtgtc 180  
 aaagggtggtg gtcattgtgc acaaatttat gcaattcgac agtcaattgc taaagttttg 240  
 gtggcctatt accagaaaaa cgtggatgag caaagcaaga aagaattgaa ggatcaactt 300  
 gttgcttatg atcgtaatat gcttgttgcc gatccgagac gtcacgagcc aaagaagttt 360  
 ggaggacctg gtgctcgtgc tcttattcag aaatcttatt gtttaagaagt atgaaattat 420  
 aaaattgtgt gttacgaatt aattgttatt ttgttgggat aaatntgaat a 471

<210> 117  
 <211> 593  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 117  
 gaattcaaaa aatattaaaa ttgtttaata taatttctaa aatgaagcca aaggttggaa 60

## AKK110P1

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ttaacggatt tggacgtatt ggacgtcttg ccctgcgtgc agcggtcgag aaggatactg 120
tccaagttgt ggctgtcaat gaccggttca ttgatcttga ctatatggtc tatatgttta 180
actatgattc caccacacga cgctttaaag gaaagattca agcaagcaat ggaaatttgg 240
tagttgagaa ggaggggaaag tctactcata ctatcaaagt tttcaacttc aaagaacctg 300
aaaagattga ctgggcaggt tctggtgctg attttgttat tgagtcgact ggagttttta 360
ctactaccga gaaagcttct gctcacttga agggcgagc caagaaagtg gttatctccg 420
ctccatctgc tgatgctcca atgtttgtgg ttggtgttaa tgaggacaaa tatgatcctt 480
ccaagcatca tatcattagt aatgcttcct gcaactactaa ttgtcttgct cctcttgcca 540
aggttataaa tgacgagttt ggcataattg aaagttgaat gactactgga cac 593

```

<210> 118  
 <211> 576  
 <212> DNA  
 <213> Meloidogyne incognita

```

<400> 118
gaattccgag tttttttttt ttttttttaa aacaaaaatt aaaagattta tcgccatcct 60
ttgccagcca ttgcccgcg atttttttgt gcacaataaa tttttttgta atttttgggg 120
tgagggggaa gtaaaatgaa agaagggaga gagatatgaa ttggagggtt tttgtttaa 180
ataaattttt tttcttgaa aattcttccc gtttctgagc tttttctct ttttcaatt 240
ttcgttttgtc gaaatactaa actttacaat ttggttaggt tctattttgtg aaacataaat 300
atctccatta tcgctgattg caagggcag ggcgttttcg agacccttg caaagctatt 360
agcccttctt gtgttcatat ccattacgaa aacttgggat tctaattgac tgccttgatc 420
ttgattggtg acgccgacga ggaagtgttc tttctctcg atagcaaaga ctgcaccaat 480
attttcagcc ttgtgaaga aagtgcctgt ggggacgtaa gcacgtctat gttggtgttg 540
agcgcttct aatccagcag aaaagcattg aatacg 576

```

<210> 119  
 <211> 559  
 <212> DNA  
 <213> Meloidogyne incognita

```

<400> 119
acgcagagta agttgagatc ttcaataagg gttagagagt gtggtacgag gaattctcca 60
tttttgggtg ttccactgga gtcaggcttc ccgaattgac tgagcaattt cccatccttg 120
tcaaacttca ttattcggct attacagtaa ccatctgccg cgaaaaactc tctgtactg 180
gcaatagcaa cgtctgtagg tttgcaaaaa tgtttgcatt ctgtccctgg aacaagcttt 240
tcgcccacac tcataattaa tttaaaatcc ttgtcaagtt tgtggacttg atgacttcca 300
acgtcagtaa cccaactatt gccgtgggca tcgattgtta gtccatgagg catgtaaaac 360
atgctttttc cgtattcttc caagactgcc cctgattccg tgtctataac agcaattgtt 420
gtgtttgaaa tgatgccag ggaatcgttt aggtggttgt tctcatcaaa cgaaaattca 480
tcccaaacctc tgtcagatcg gtgaaaaaga acaagtcgat tcaatggatc caatgcaata 540
cccggagctt gcccaatat 559

```

<210> 120  
 <211> 366  
 <212> DNA  
 <213> Meloidogyne incognita

```

<400> 120
tttaagaatt ttttaaaaat taaaacttgg actagatttt aataaaatgt cagctccacg 60
tagtgttgct agcgggtgtg gtgctgctgt tatgaataag caagcaagta aatacaatga 120
agttgaagga gaactccttc ttaattggat taagaaagtg acaggcgaaa atattgctat 180
aaacggaact agggaaaatt ttgtgaaaca attgaaagat ggaactctgc tctgcaaat 240
tgctaacaaa attgtgccaa attcaatcac aaaggcacag gcaaaaaccg acagcacatt 300
ccaatatatg agcaatttgg agctgttctt aacatttatt tcaagccaag gagtccctag 360
ggagga 366

```

<210> 121  
 <211> 661  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 121



## AKK110P1

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ttagttgaat ctcgtgacct ctactctgtt tgtatgacat taaattctct tggccgcatt 60
ttggaacgct aaggaaaaac tcattccagag caggttaagt cgtcagaaat tcttaatttg 120
ggtagctggag accaagtgcg ctttcgtgtt taaagatggg aaattgaaag aatttttggt 180
aaacataata aaaagacatt ttatggcaat aaaaaaatgt caaaaaagct tgtcttttaa 240
atattttggc aaaacatttt actttcacaa aatttttaaa taaatttatg aagattgttc 300
cgtcactttc atcatttccg atcgaccttt gttgttttct aagttcgttg gccaaagaaa 360
ggatatgtaa aattgaatta tgaataaaaa taaatcactc aatcagaggc attgttagtc 420
tctcactttc tctcttttac ccattggcta accagcttta aggatttttt ccataagttc 480
aaggtgtacg taaatcgaat accgactgtg gtatcttaat ttttccatga aattctccaa 540
taaaaaaaa tttttttat ttttttcca taatgctatc tatattttt gcttttaac 600
ttttttggt atcaggcttt aaaatagtaa atatacttat attaataatt tatttccttt 660
a

```

<210> 122  
 <211> 173  
 <212> DNA  
 <213> Meloidogyne incognita

```

<400> 122
ggagagtttt tcgtggcaga tggttactgt aatagtcgaa taatgaagtt tgacaaggat 60
gggaaattgc tcagtcaatt tgggaagcct gactccagt aaacacccaa aaatggagaa 120
ttcctgtac cacactctct aaccctcatt gaagatctca acttactttg tgt 173

```

<210> 123  
 <211> 584  
 <212> DNA  
 <213> Meloidogyne incognita

```

<400> 123
cgcattcaat gcttttctgc tggattagaa ggcgctcaac accaacatag acgtgcttac 60
gtccccacag gcactttctt cacaaggct gaaaatattg ggcgagctt tgctatccga 120
gagaaagaac acttcctcgt cggcgctcacc aatcaagatc agggcagtc attagaatcc 180
caagttttcg taatggatat gaacacagga agggctaata gctttgctaa ggtctagaa 240
aacgcccatt cccttgcaat cagcgataat ggagatattt atgtttcaca aatagaaccc 300
aaccaattg taaaatttag tatttcgaca aacgaaaatt gagaaaaaaa aaaaaaaagc 360
tcagaaacgg gaagaatttt caagaaaaaa tttttttacc aaacaaaaaa cctccaattc 420
atactctccc ctcttttcat ttttcttccc ctttctcccc aaaaattaca aaaaatttta 480
ttgtgcacaa aaaaatgggc gggcgggcga atggctgggc aaaggatggc gataaatctt 540
ttaattttg aaaaaaaaaa aaagaattcg aatttatagg ccta 584

```

<210> 124  
 <211> 650  
 <212> DNA  
 <213> Meloidogyne incognita

```

<400> 124
gtttaagaca attaaaacgt ttattttcta caatcaaac aaatatggct gttcctcccc 60
atgttatcga gaagatcgag gctgggtaca aaaagtgcga ggaggaccg gagtgaagt 120
ctcttctcaa gaagtacttc acgaaggaa ttatggacca gtgtaaagg ctcaaaaacta 180
agcttggatg gaacttgctt gatgtgatcc actctggagt tgcgaatctc gatagcggg 240
ttggtgttta tgcgcctgat gctgagtctt acactctctt caaacgcgtt ttgacctga 300
ttattcagga ttaccacaat ggatttggac ctgaccagaa gcagccgcaa actgacttgg 360
gtgagggaaa gactcagctt ttgcctgatc tggatcctga gggtaaattc atcaactcga 420
ctcgtgttcg atgtgggcgt tctcttcagg gatatccgtt caatccgtgc ttgactaaag 480
agaattatc ggaaatgcat gacaaagtta aagggtttt tgagcagctt aagtctgatg 540
ctgagcttgg tggcacctat tatcctttgg agggaatgac caaagaggtt caaactcaat 600
tgatcaagga tcacttcttc ttcaaagaag gagaccgctt tttgcaagct 650

```

<210> 125  
 <211> 1013  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 125

## AKK110P1

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ttaaattttct	tctttatttt	ttaaaaaatt	atttcttaaa	tttattcttc	tcctcttcgt	120
gttttgatc	aaataattaa	attttaaatt	atttaaacag	ctacacgagg	cctcagcctc	180
ccccgttgca	ttcaaatgg	tcggcacggt	tggcgatgat	aattttattt	tttaggtaat	240
tttggtgaga	aaatattttt	aaaggtaata	atgtcctttt	ggacaattaa	aaaaaaactc	300
gaggagagag	tgaatatttt	tacaaattat	ttgaagagca	gccagcctat	tgttatcaac	360
aaaaaacctt	caaaatgcca	gaaaatgatt	atgatgagga	ggaggcgcca	aacgccacga	420
tggaacaaca	ggtagcttca	ggtggacagc	caaaacgctg	ttggaaaatg	gacattatcc	480
cagctgcgcc	agactgatgg	tataattcca	tcccaggccg	gttggaaaca	gggagactcc	540
caaaagtgtg	tgaccaattt	tggtactcca	cgtaacacaa	caaccaaaat	tcgtgctgaa	600
tgcccttgctg	atgtgcctga	agaaaattgct	cttaaaaagtc	acggtgaagt	acgcctccaa	660
tccggtacta	accgittttg	ttcgcagaag	ggaatggttg	gatttggtac	tggaactgac	720
ttatgcagag	aaggagtggt	tgtgagtcaa	gacccagccg	atttatagcc	cctcccagaa	780
gagataatcc	gtgctagcga	tggaaattggt	cgctctcaat	ccggtacca	caaattcgac	840
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accaaaccatc	cggaaataca	ccacgaagtt	aacattgacc	aaagcgaaat	tcctttgcaa	960
tctggtacaa	acaaattcgc	atcccaaaag	ggaatgacca	gcttcggtac	aaa	1013

&lt;210&gt; 126

&lt;211&gt; 80

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 126

tggtggacac	tgctcaccca	gaatacagtc	acgaaagcag	catcgatcaa	acgagcattc	60
cttaccaa	gggatcaaat					80

&lt;210&gt; 127

&lt;211&gt; 585

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 127

agggaaatgac	ttgcttttga	cagccacggt	gggaggtgct	tgacccgagc	attagctacc	60
agaaccgtaa	atcacaaagg	atggtccgtc	tccaatccgg	aacaaaccgg	gtcgcctcgc	120
aagcgggcat	gacaggtttt	ggaactccaa	ggaacacaac	atacagggcg	gagtcctggcg	180
aacttccata	cgaagatatg	aagaagtcag	aaacgataat	tccatcccag	gccggttga	240
ataagggaga	ctctcaaaag	ttgatgactg	gatttggtac	tcctcgtgac	gttaaaggca	300
aacatttgaa	gcgtatttgg	gagttggaaat	acccagagga	ggctgaaatt	tcgttgatc	360
gactttaaag	gaattttaga	agagaagaaa	gaaaagagaa	atttagtga	aggaaggcaa	420
cgacatttga	ctctacaatt	gacacacacc	ttttcacaca	tttacaataa	acattaaaaa	480
aaaatttttt	ttggcttttt	ggcttgctcc	tattttttcc	ccccatcatt	ctccctattc	540
tctcatttgg	atgcaaaactg	gaattttaaa	aaaaaaaaaa	aaaaa		585

&lt;210&gt; 128

&lt;211&gt; 287

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 128

catctggaga	aacgttgagg	caatacatcg	ttattggccg	taaacttcct	acagagaatg	60
agccaaatcc	aaaactttac	aaaatgcaaa	tttttgccag	taatcatggt	gttgctaaat	120
cgcgtttctg	gtactttact	agtatgttgc	gtcgtgttaa	gaagactaac	ggagagattg	180
tttcgtgtca	ggagggtttt	gaaaagaaga	taggctctgt	aaagaattat	ggaatttggc	240
ttcgttatga	ctctgaacc	ggtcatcaca	acatgtaccg	tgaatac		287

&lt;210&gt; 129

&lt;211&gt; 175

&lt;212&gt; DNA

&lt;213&gt; Meloidogyne incognita

&lt;400&gt; 129

gctgtcactc	aggcttatcg	cgacatgggt	gctcgtcact	gtgctcaagc	cgatcgaatc	60
caaataatca	aggttcaacc	gatcaaggct	gccgatttga	aacgtactgg	agttaaacag	120

AKK110P1

ttccacaact cttcaatcaa gtttcctttg ccgcatcgtg tgaatgacaa acgtc 175

<210> 130  
 <211> 599  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 130  
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 aaagggttcac cttctaagta aggattgtag cggctgtatg attgatgttg cttttgttgg 120  
 ggagcaatag aacgcttgcg tcgccgaggc tcctcagccc tagtaacgtg aaatttcttt 180  
 gcaatcatcg atttgtgtag tccatttttg gctaagacct gttctaagtc ttgttcatat 240  
 tgttcagaat tgctttttga ttgacagtta aacatgtgtt cttggtcaca aaggcattgc 300  
 tgattggcct ggtagctacg cgagaaatcg gcggtgttat caaactcctc caaacatcca 360  
 tctcgactgg agtatcccac agggcagggg tttggagggt cacaatatgc tggcaaaaaca 420  
 ttgtcactct taatctcttg gcggtgtgaa aattcagatt ctggatggag ttgttggctt 480  
 ccttcaccgg cactctctgt cataaattta tgtccaaacg caatggggccc ggaagcactt 540  
 tcaatgtcac gagaaatcaa gtcgattaat tgtgaatgcg gaaatatagg ctccccaga 599

<210> 131  
 <211> 466  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 131  
 gaagattgga tttattggcg ctggaaagat ggacacaggca ttggccagag gactaataaa 60  
 ttctggacgt tatccttcac aaaatttgat ggctagtgtc cctaagactg atgtctcttt 120  
 attggaggat tgcaagaggc ttgggagtaa tacagcacat gataatgcac aagttgctcg 180  
 tgaatatgat gtggtgatta tagcaggtaa accaactatt gtgtctaaag ttgtctcgga 240  
 aattgcacca gccatccgcc gagatcatgt acttatttct atagcattgg gcatccaccat 300  
 acgctacatt gagcagtaat tgacttcaga atcccgaatt gttcgtgtaa tgcagatac 360  
 tcctgtagggt ggtaggagca ggctgctgca gccatatatc attgggatca gcattgtcag 420  
 gatagggtgat gcccagatag ttcaagatct tctgataacg ctggggg 466

<210> 132  
 <211> 266  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 132  
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 tggaaattgga agcgccgttg aagattttgtg gcgatattca cggtaaatc aacgaccttt 120  
 tgcggctttt tgaatatgga ggttttccgc ctgaagcgaa ttatttattt ttgggtgatt 180  
 atgtggatag aggaaagcag agcttggaga cgatttgtt gctgttgcc tacaagatca 240  
 aatccccga aaattctttt tgctga 266

<210> 133  
 <211> 308  
 <212> DNA  
 <213> Meloidogyne incognita

<400> 133  
 tctatcaacc gaatatatgg attttacgat gaatgcaaac gcagattttc tataaaattg 60  
 tggaaaacat ttactgattg cttcaattgt ctgccaattg ctgctgtgat cgatgagaaa 120  
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&lt;213&gt; Meloidogyne incognita

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AKK110P1

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&lt;213&gt; Globodera rostochiensis

&lt;400&gt; 12

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&lt;210&gt; 13

&lt;211&gt; 161

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

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&lt;211&gt; 306

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

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&lt;211&gt; 261

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

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&lt;210&gt; 16

&lt;211&gt; 151

&lt;212&gt; DNA

&lt;213&gt; Globodera rostochiensis

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&lt;210&gt; 17

&lt;211&gt; 306

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